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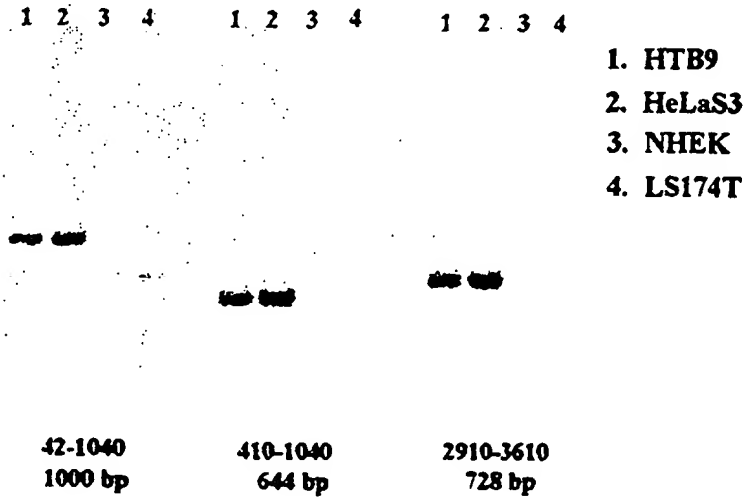

(57) Abstract

Methods of screening for or treating cancer are disclosed. The screening methods are based on the detection of an antigen, or a nucleic acid molecule encoding the antigen, found by the present invention to be associated with the presence of cancer. Preferred embodiments of the methods include detection of the antigen based on immunological properties, physical properties, enzymatic properties and combinations thereof, or detection of a nucleic acid molecule encoding the antigen based on nucleic acid amplification.

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METHODS AND COMPOSITIONS FOR SCREENING FOR OR MODULATING A TUMOR ASSOCIATED ANTIGEN

TECHNICAL FIELD

The present invention is generally directed toward screening for or modulating a tumor associated antigen. The invention is more particularly related to detecting a complement Factor H-related protein, or a nucleic acid molecule encoding such a protein, associated with the presence of cancer, and to modulating the presence or activity of such a protein.

BACKGROUND OF THE INVENTION

The detection of new tumors or the recurrence of tumors remains an unfulfilled goal of humankind, despite enormous expenditures of both financial and human resources over the last twenty-five plus years. A number of cancers are treatable if detected at an early stage, but unfortunately go undetected in many patients for lack of a reliable screening procedure. For illustrative purposes, background for a particular cancer, bladder cancer, is described in more detail and is representative of cancers in need of new approaches, which the invention disclosed herein provides.

Bladder cancer is the fifth most common cancer in the United States. The American Cancer Society estimated that a total of 52,000 new cases would be detected in 1994 and that there would be 10,000 deaths resulting from this disease. Bladder cancer is more common in men than in women by a ratio of approximately three to one and has been shown to be highly associated with smoking as well as exposure to certain dyes. Carcinoma of the urinary bladder is the fourth most common malignancy among American men, and the eighth among women. Transitional cell carcinoma (TCC) is the most common type of bladder cancer representing greater than 90% of all cases. The remaining cases are squamous cell carcinomas (7%), adenocarcinomas (2%), and undifferentiated carcinomas (1%).

The diagnosis and management of TCC is often performed as follows. The patient presenting with such symptoms as hematuria or dysuria in the absence of infection undergoes a cystoscopy at which time the tumor is visualized. Although this

procedure is invasive and unpleasant, it is highly accurate in predicting malignancy and is, thus, considered the gold standard. Urine cytology (i.e., the identification of tumor cells in voided urine) is also performed, and the combined results of the two methods may lead to an increase in sensitivity over that of cystoscopy alone. This is due to the fact that cytology occasionally allows detection of tumors which are not visible during cystoscopy, for example, flat tumors of the bladder (TIS) or those in the upper end of the bladder or the upper urinary tract.

Transurethral biopsy and resection are then usually performed with this procedure removing the apparent lesion as well as providing information as to the grade and stage of the tumor. The tumor is typically graded from G0 to G4 in decreasing state of differentiation. As with most cancers, the less differentiated the tumor the more aggressive the disease. With respect to stage or extent of invasion, TCC's of the bladder may be classified as superficial papillary (Ta and T1), muscle invasive (T2 and greater), or the relatively uncommon tumor in situ (TIS). The extent of invasion dictates the type of therapeutic approach employed and the follow-up procedures to monitor for disease recurrence.

Individuals with invasive TCC (Stage T2, T3, and T4) typically have poor prognoses. They are usually treated by radical cystectomy; however, in some cases the patient is unable to tolerate this surgery and is treated by radiation therapy or chemotherapy instead. This latter subgroup is monitored for disease recurrence by cystoscopy and urine cytology.

Approximately 75% of TCC patients are initially diagnosed as having either Ta or T1 disease. In part because bladder cancer is multifocal, initial resection and treatment of these patients is curative in less than half of the cases. Although patients presenting with Ta TCC usually recur, their tumors tend to be low grade, and only 10-15% of the tumors will progress to muscle invasive disease. In contrast, T1 patients will progress 30-50% of the time. Superficial TCC is usually treated by transurethral resection, intravesical therapy, or fulguration, and follow-up is usually by cystoscopy and voided urine cytology.

As mentioned above, current practice includes a preliminary diagnosis of TCC by cystoscopy and urine cytology, confirmatory diagnosis and staging and grading by biopsy, and routine follow-up of superficial and some invasive TCC by cystoscopy and urine cytology. Recurrence, especially within the first 12 months, is common, even when tumors have been diagnosed and treated prior to invasion of the bladder muscle. Therefore, patients with superficial TCC are typically monitored every three months for the first two years and, if there is no recurrence, every six months during the following year. Because cystoscopy is invasive and unpleasant and because urine cytology, although highly specific, is of variable reliability in detecting recurrence, there is a significant need for alternative diagnostic approaches.

Accordingly, there is a need in the art for a non-invasive diagnostic method with reliability in detecting occurrence or recurrence. The present invention fulfills this need and further provides other related advantages.

SUMMARY OF THE INVENTION

Briefly stated, the present invention provides a variety of methods and compositions for screening for cancer, and for treating tumor cells. The screening methods and compositions may be used on a one-time basis when cancer is suspected or on a periodic basis, e.g., to monitor an individual with an elevated risk of acquiring or reacquiring cancer.

In one aspect, the present invention provides a method of screening for a cancer comprising the step of detecting the presence of a tumor-associated human complement Factor H-related antigen or a nucleic acid molecule encoding the antigen, the nucleic acid molecule characterized by the ability of the nucleic acid molecule to hybridize under moderate stringency with the primer pair 42M/1040RT (SEQ ID NO:10 and SEQ ID NO:17, respectively) or the primer pair 2910M/3610RT (SEQ ID NO:18 and SEQ ID NO:19, respectively).

Preferred embodiments of the methods for either aspect include detection of the antigen based on immunological properties, physical properties, enzymatic properties or combinations thereof, and detection of a nucleic acid molecule encoding the antigen by amplification of the molecule.

In a related aspect, the present invention provides a method of treating a tumor cell comprising the step of modulating a tumor-associated human complement Factor H-related antigen or a nucleic acid molecule encoding the antigen, the nucleic acid molecule characterized by the ability of the nucleic acid molecule to hybridize under moderate stringency with the primer pair 42M/1040RT (SEQ ID NO:10 and SEQ ID NO:17, respectively) or the primer pair 2910M/3610RT (SEQ ID NO:18 and SEQ ID NO:19, respectively).

In another aspect, the present invention provides agents that modulate a tumor-associated human complement Factor H-related antigen or a nucleic acid molecule encoding the antigen. In one embodiment, an agent is provided that modulates a tumor-associated human complement Factor H-related antigen or a nucleic acid molecule encoding the antigen, the nucleic acid molecule characterized by the ability of the nucleic acid molecule to hybridize under moderate stringency with the primer pair 42M/1040RT (SEQ ID NO:10 and SEQ ID NO:17, respectively) or the primer pair 2910M/3610RT (SEQ ID NO:18 and SEQ ID NO:19, respectively), for use as a medicament to treat a tumor cell. In another embodiment, a composition is provided comprising an agent that modulates a tumor-associated human complement Factor H-related antigen or a nucleic acid molecule encoding the antigen, the nucleic acid molecule characterized by the ability of the nucleic acid molecule to hybridize under moderate stringency with the primer pair 42M/1040RT (SEQ ID NO:10 and SEQ ID NO:17, respectively) or the primer pair 2910M/3610RT (SEQ ID NO:18 and SEQ ID NO:19, respectively), in combination with a pharmaceutically acceptable carrier or diluent. In another embodiment, the present invention provides for use of an agent that modulates a tumor-associated human complement Factor H-related antigen or a nucleic acid molecule encoding the antigen, the nucleic acid molecule characterized by the ability of the nucleic acid molecule to hybridize under moderate stringency with the primer pair 42M/1040RT (SEQ ID NO:10 and SEQ ID NO:17, respectively) or the primer pair 2910M/3610RT (SEQ ID NO:18 and SEQ ID NO:19, respectively), for the manufacture of a medicament for the treatment of a tumor cell.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the gel electrophoresis of the first-step RT-PCR amplification products, with lanes 1 to 10 beginning at the right side of the gel as lane 1. Lane 1: X44.1 mRNA; Lane 2: HTB-5 mRNA; Lane 3: HTB-9 mRNA; Lanes 4, 5, 6 same targets as 1, 2, 3 respectively except the RT primer was OligodT_{16}. Lanes 7 and 10, DNA molecular weight markers at 2000, 1500, 1000, 700, 500, 400, 300, 200, 100, and 50 base pairs. Lanes 8 and 9 are PAW 109, the kit positive control, at the expected size of 311 base pairs.

Figure 2 shows the gel electrophoresis of the second-step PCR amplification products, with lanes 1 to 10 beginning at the right side of the gel as lane 1. Lane 1: X44.1 product (reaction 1, lane 1) with primers 753RT and 412M; Lane 2, HTB-5 product (reaction 1, lane 2) with 753RT and 412M; Lane 3, HTB-9 product (reaction 1, lane 3) with 753RT and 412M. Lane 4, PAW109 (reaction 1, lane 8) product with 753RT and 42M primers; Lane 5, X44.1 product with 753RT and 42M primers; Lane 6, HTB-5 product with 753RT and 42M primers; Lane 8, HTB-9 product with 753RT and 42M primers. Lanes 7 and 11, DNA molecular weight markers. Lane 9, PAW109 product (reaction 1, lane 8) with DM152 and DM151 primers; Lane 10, PAW109 product (reaction 1, lane 8) with 753RT and 412M primers.

Figure 3 shows stimulation by MAb X52.1 of the complement-mediated lysis of rabbit red blood cells. The extent of lysis is shown after 45 and 117 minutes with complement alone and in the presence of X52.1 at concentrations of 10 nM and 30 nM.

Figure 4 shows stimulation by MAb X52.1 of the complement-mediated lysis of HL-60 human myeloid cells. The extent of lysis is shown after 120 minutes (a) with complement alone, (b) in the presence of X52.1 at a concentration of 10 nM, and (c) in the absence of complement.

Figure 5 shows the gel electrophoresis of amplification products resulting from RT-PCR performed with three primer sets derived from human

complement Factor H (lanes 1 to 10 beginning at the left side of the gel with the left side set of numbers 1-4 on the Figure representing lanes 1-4, the middle set of numbers 1-4 representing lanes 6-9 with lane 5 preceding, and the right side set of numbers 1-4 representing lanes 11-14 with lane 10 preceding). Lane 1: HTB-9 product with primers 1040RT and 42M; Lane 2: HeLaS3 product with primers 1040RT and 42M; Lane 3: NHEK product with primers 1040RT and 42M; Lane 4: LS174T product with primers 1040RT and 42M; Lane 6: HTB-9 product with primers 1040RT and 410M; Lane 7: HeLaS3 product with primers 1040RT and 410M; Lane 8: NHEK product with primers 1040RT and 410M; Lane 9: LS174T product with primers 1040RT and 410M; Lane 11: HTB-9 product with primers 3610RT and 2910M; Lane 12: HeLaS3 product with primers 3610RT and 2910M; Lane 13: NHEK product with primers 3610RT and 2910M; Lane 14: LS174T product with primers 3610RT and 2910M; Lanes 5 and 10: DNA molecular weight markers.

Figure 6A shows a partial DNA sequence from clone pRBB9FH410 (SEQ ID NO:22) and Figure 6B the corresponding deduced amino acid sequence (SEQ ID NO:24), as compared to the DNA and amino acid sequences for human CFH (SEQ ID NOS: 21 and 23 respectively).

Figure 7A shows three partial DNA sequences from clone pRBS3FH2910 (SEQ ID NOS: 26-28) and Figure 7B the corresponding deduced amino acid sequences (SEQ ID NOS: 30-32), as compared to the DNA and amino acid sequences for human CFH (SEQ ID NOS: 25 and 29, respectively).

Figure 8A shows two partial DNA sequences from clone pZS3FH2576 (SEQ ID NOS: 34 and 35) and Figure 8B the corresponding deduced amino acid sequences (SEQ ID NOS: 37 and 38), as compared to the DNA and amino acid sequences for human CFH (SEQ ID NOS: 33 and 36, respectively).

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is directed, in one aspect, toward methods of screening for cancer. As disclosed in the present invention, a protein antigen has been found to be associated with the presence of cancer ("tumor-associated") and found to survive in detectable concentrations in samples from warm-

blooded animals, such as humans. The present disclosure describes, for example, the purification of a tumor-associated antigen from cancer patients, the generation of antibodies to the antigen, the characterization of the antigen by physical and biological properties, the development of immunoassays and non-immunoassays for the detection of the antigen or a nucleic acid molecule encoding the antigen, the evaluation of samples from normal individuals and cancer patients, demonstration of the production of the antigen by cancer cells, the determination that the antigen corresponds to protein products related to human complement Factor H, and the inhibition of biological activity of the antigen.

A wide variety of cancers may be screened. Representative examples of such cancers include urogenital, renal, head/neck and lung. Urogenital cancers include bladder, cervical and prostate. Head/neck cancers include cancers of the oral cavity, mouth and esophagus. As used herein, the term "screening for" includes detecting, monitoring or diagnosing. It will be evident to those in the art that if one wishes to screen for a particular type of cancer, this choice will guide the selection of a particular source of cell, tissue or sample to be tested. A sample in general may be a liquid or solid (e.g., cellular) sample taken from a tissue or organ, or after having been in contact with a tissue or organ. For example, a prostate sample includes a sample taken from a prostate or after having been in contact with a prostate. Representative types of prostate samples include prostate scraping and prostate tissue biopsy. A head/neck sample includes a sample taken from a head/neck or after having been in contact with a head/neck. Representative types of head/neck samples include swabs, scrapings and tissue biopsy of the oral cavity and esophagus. A lung sample includes a sample taken from a lung or after having been in contact with a lung. Representative types of lung samples include bronchial wash, sputum and tissue biopsy of the lung. A bladder sample includes a sample taken from a bladder or after having been in contact with a bladder. Representative types of bladder samples include urine, bladder wash, bladder scraping and bladder tissue biopsy. Urine may be voided or pre-voided (i.e., in a bladder). Urine may be removed from a bladder by using, for example, a catheter or a needle. A cervical sample includes a sample taken from a cervix or after having been in

contact with a cervix. Representative types of cervical samples include cervical swab. cervical wash, cervical scraping and cervical tissue biopsy. Pretreatment of a sample may be desirable. For example, in the case of urine samples neutralizing the pH with buffer may be desirable.

The detection, isolation, characterization and identification of a protein antigen present in specimens derived from patients with cancer, but absent in specimens from normal individuals, indicates that this antigen is either a product of the cancer cells or is for some other reason present in specimens from these patients. If the antigen is expressed by cancer cells, it may be present in the supernatants taken from cultured human cancer cell lines at levels adequate to be measured by enzyme immunoassay specific for the antigen. cDNA derived by reverse transcriptase-polymerase chain reaction (RT-PCR) amplification from mRNA isolated from the same cancer cells can be used as well to provide evidence for expression of the gene which encodes for a product which is identical or very similar to the identified antigen. As disclosed herein, both types of experimental approaches confirm the expression of the antigen by cancer cell lines (e.g., bladder, cervical, renal and prostate cancer cell lines).

The tumor-associated protein antigen of the present invention has been determined, by sequence comparisons, surprisingly to be human complement Factor H-related. As cancer cells may produce more than one form of the protein, as used herein the term "human complement Factor H-related" refers to the human complement Factor H protein and variants thereof. The variants may be the result of mutations, alternate splicing or recombination events that alter nucleic acid molecules encoding human complement Factor H. In general, the amino acid sequence identity between a human complement Factor H-related protein from a tumor cell and human complement Factor H will be at least about 50%. More typically, the amino acid sequence identity will be at least about any integer from (and including) 50% to 100%, such as at least about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% identity. Variants that are nearly identical to human complement Factor H have at least about 85% or 90% identity. As used herein, amino acid sequence "identity" is determined by the alignment of amino acid sequences and establishment of identical amino acid residues using the

program GeneJockey II (1993) for Macintosh (Philip L. Taylor, published by Biosoft, Cambridge, UK). The program is run in the amino acid homology mode, using program default parameters. In the comparison of two sequences aligned by the program, the percent identity is calculated only for those positions where there is an amino acid residue present in both of the two sequences. In addition, a nucleic acid molecule encoding for a human complement Factor H-related protein will typically hybridize under moderately stringent conditions to one or the other or both of two primer pairs (42M/1040RT or 2910M/3610RT), as described below. This reflects conservation of certain sequences (disclosed herein) for tumor-associated human complement Factor H-related antigen. A protein may generally be identified as a tumor-associated human complement Factor H-related antigen based on the ability of a nucleic acid molecule encoding the protein to hybridize under moderately stringent conditions to one or the other or both of two primer pairs (42M/1040RT or 2910M/3610RT), as described below. Based on the disclosure herein, in combination with the methodologies known in the art, it will be evident to those in the art whether a protein is a tumor-associated human complement Factor H-related antigen, or whether a nucleic acid molecule encodes such a protein.

The antigen may be isolated in substantially pure form. Briefly, for example, urine samples of bladder cancer patients are clarified (e.g., by centrifugation) and concentrated (e.g., by hollow fiber concentrator). The concentrated sample is chromatographed on heparin agarose, and bound material eluted using a linear buffered NaCl gradient. Pooled fractions are concentrated. Purity can be assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis ("SDS-PAGE") with appropriate protein stains. Alternatively, the antigen may be purified using an antibody against the antigen, as described for example below.

Following isolation of antigen, the polypeptide constituents may be identified. Typically, polypeptides are resolved by separation (e.g., by gel electrophoresis) under denaturing conditions (e.g., sodium dodecyl sulfate). Approximate molecular weights of polypeptides are assigned by comparison of their mobility to the mobility of polypeptides of known molecular weights on SDS-PAGE.

Isolated antigen yields from certain cancers, for example, a band with an apparent molecular weight of approximately 151,000 on SDS-PAGE under reducing conditions (i.e., in the presence of DTT which is 1,4-dithiothreitol). Rather unusually, this band exhibits a lower apparent molecular weight (of approximately 138,000) on SDS-PAGE under non-reducing conditions (i.e., in the absence of a reducing agent). This somewhat anomalous electrophoretic behavior provides a convenient means for identifying the antigen.

Purified antigen, partially purified antigen or biological samples containing antigen may be used to produce antibodies that specifically bind to the antigen. Antibodies that specifically bind are those with an affinity of about 10^6 liters/mol or greater. Either polyclonal antibodies or monoclonal antibodies may be generated. Polyclonal antibodies may be produced by immunization of an animal and subsequent collection of its sera. It is generally preferred to follow the initial immunization with one or more booster immunizations prior to sera collection. Monoclonal antibodies are generally produced by the method of Kohler and Milstein (*Nature 256*:495-497, 1975; *Eur. J. Immunol. 6*:511-519, 1976). Briefly, the lymph nodes and/or spleens of an animal injected with antigen in pure or impure form are fused with myeloma cells to form hybrid cell lines ("hybridomas" or "clones"). Each hybridoma secretes a single type of immunoglobulin specific for the antigen and, like the myeloma cells, has the potential for indefinite cell division.

Antigen in pure or impure form ("immunogen") is used for the immunization. Preferably, the animals are immunized with at least 100 ng each of the immunogen, most preferably greater than 500 ng each. For immunization, the immunogen may be adsorbed to a solid phase matrix, preferably to nitrocellulose paper. The paper is then introduced into the animal. Techniques for introduction of the adsorbed antigen preparation include implantation (U.S. Patent No. 4,689,220) or solubilization of the solid phase and injection of the solubilized material (Knudsen, *Anal. Biochem. 147*:285-288, 1985). The solid phase matrix may be solubilized in an appropriate organic solvent (e.g., DMSO) and either mixed with adjuvant or saline, or injected directly.

Alternatively, the immunogen may be injected in the absence of a solid matrix and/or adjuvant. Injection or implantation may be intraperitoneal, intra-foot pad, subcutaneous, intramuscular or intravenous, but preferably intraperitoneal. The animals may also be injected with antigen complexed with adjuvant, such as Freund's adjuvant. Single or multiple booster immunizations are used. Between one and seven days prior to the fusion date, preferably on days one through four, intravenous injections of the immunogen may be given daily.

Between one and seven days, preferably four days, after the administration of the final booster immunization, spleens or portions thereof are harvested from the immunized animals. At this time, the lymph nodes may also be harvested and included in the cell preparation. The harvested organs are minced using techniques which disrupt the structure of the organ, but which are not detrimental to the lymphocytes. The organs are preferably minced with scissors, passed through a mesh screen and mixed with growth medium to enrich the preparation for lymphocytes. The minced and strained tissue is harvested by centrifugation, then mixed with growth medium to form a cell suspension. The red blood cells may be lysed by adding a hypotonic or hypertonic solution to the cell suspension. A preferred method for cell lysis is to add distilled water to the suspensions and quickly return the suspensions to an isotonic state with a hypertonic sodium chloride solution. Any remaining tissue may be removed by filtration through gauze.

The harvested cell suspension is then mixed with a myeloma cell line, preferably one which is syngeneic with the immunized animal. Myeloma cell lines from various species are widely available through, for example, American Type Culture Collection (ATCC), Rockville, Maryland. Myeloma cell lines commonly used include P3X63Ag8 (ATCC TIB 9), SP2/0-Ag14 (ATCC CRL 1581), FO (ATCC CRL 1646) and 210-RCY-Agl (Galfre et al., *Nature* 277:131, 1979).

The myeloma cells are cultured in an appropriate mammalian cell growth medium, a variety of which are generally known in the art and available from commercial sources. Mammalian cell lines are routinely grown between 36°C and 40°C under conditions which maintain an optimal pH between 6.0 and 8.0, preferably

about pH 7.2. pH may be maintained through the use of a variety of buffer systems known in the art. A preferred buffer system involves growing the cells in a bicarbonate buffer in a humidified incubator containing CO_2, preferably about 7% CO_2.

The fusion between the lymphocytes from the immunized animal and the myeloma cells may be carried out by a variety of methods described in the literature. These methods include the use of polyethylene glycol (PEG) (Brown et al., *J. Biol. Chem.* *255*:4980-4983, 1980) and electrofusion (Zimmerman and Vienken, *J. Membrane Biol.* *67*:165-182, 1982). An electrofusion generator is commercially available from Biotechnologies and Experimental Research, Inc., San Diego, California.

Following the fusion, the cells are plated into multi-well culture plates, preferably 96-well plates. A reagent which selectively allows for the growth of the fused myeloma cells over the unfused cells is added to the culture medium. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. Other selection techniques may also be used depending on the myeloma cell line chosen.

Alternative methods of producing monoclonal antibodies utilize *in vitro* immunization techniques. Lymphocytes may be harvested from lymphoid organs, such as spleen or lymph nodes, or from whole blood as peripheral blood lymphocytes. The lymphocytes are put into culture in the presence of the appropriate immunogen. Often immunostimulatory polypeptides will be added to the culture medium concurrently. At various times following the culturing of the lymphocytes *in vitro*, the lymphocytes are harvested and fused with a myeloma cell line as described above.

Other techniques for producing and maintaining antibody secreting lymphocyte cell lines in culture include viral transfection of the lymphocyte to produce a transformed cell line which will continue to grow in culture. Epstein-Barr virus (EBV) has been used for this technique. EBV transformed cells do not require fusion with a myeloma cell to allow continued growth in culture.

Thymocytes may be used as a feeder layer to condition the medium for the fused cells. Alternatively, peritoneal macrophages or non-immune spleen cells may be used as a feeder layer. Another alternative is to use conditioned medium from thymocytes or macrophages. Thymocytes may be prepared from juvenile mice less

than 8 weeks old. The thymus glands are harvested and minced using techniques which disrupt the thymus gland but are not detrimental to the thymocytes. This procedure is preferably carried out using scissors to mince the tissue, followed by passage of the tissue through a mesh screen. The minced and strained cell material is then harvested by centrifugation. Cell suspensions are made using growth medium. Any remaining connective tissue may be removed by filtration through gauze.

At an appropriate time following the day the cells are fused, the fused cells (hybridomas) are then analyzed for the production of antibody against the antigen. This "screening" can be done by a wide variety of techniques, including Western blot, ELISA, immunoprecipitation, effect on biological activity assays and immunocytochemical staining. These techniques and others are well described in the literature. (See, for example, J. G. R. Hurrell (ed.), *Monoclonal Hybridoma Antibodies: Techniques and Applications*, CRC Press Inc., Boca Raton, Fla., 1982.) Introduction of a screening procedure permits further definition of antibodies of useful reactivity. For example, antigen purified from a biological sample of a patient with a bladder cancer may be used in any of the above-named techniques to define antibodies which react, for example, to determinants which are common to patients with the disease.

Hybridomas which secrete antibodies of interest are maintained in culture. The cells are expanded in culture and at the same time may be cloned in such a manner as to obtain colonies originating from single cells. This provides for the monoclonal nature of the antibodies obtained from the hybridomas. A wide variety of techniques exist for cloning cells, including limiting dilution, soft agar cloning and fluorescence-activated cell sorting.

Once clones of cells are obtained, they are re-assayed for the production of the antibody of interest. These cells are then expanded in culture to allow for the production of larger amounts of the antibody. Methods for expansion of the cells include maintaining the cells in culture, placement of the cells in a bioreactor or other type of large-scale cell culture environment, or culturing the cells using various agar or gelatin carrier matrices. Antibodies are then isolated from the cell culture media.

Antibodies may be purified from conditioned media or ascites fluid by a variety of methods known in the art. These methods include ammonium sulfate precipitation, ion exchange chromatography (see Hurrell, *ibid.*) and high pressure liquid chromatography using a hydroxylapatite support (Stanker et al., *J. Immunol. Methods* 76:157, 1985). A preferred method for purifying antibodies from conditioned media or ascites fluid utilizes a commercially available Protein A-Sepharose® CL-4B column or Protein G Sepharose® (Pharmacia, Piscataway, NJ; Sigma, St. Louis, MO) or ABX mixed ion exchange resin (JT Baker, Phillipsburg, NJ). Antibodies may be purified with these columns using conditions suggested by the manufacturer.

As disclosed herein, the antigen which is found to be associated with the presence of cancer may be detected in a wide variety of ways, including by detecting the antigen itself or a nucleic acid molecule encoding the antigen. Methods for detecting the presence (i.e., qualitative or quantitative) of the antigen include those based on its physical properties, immunological properties, enzymatic properties and combinations thereof. For example, regarding physical properties, the antigen's unique polypeptide mobility on SDS-PAGE under reducing and non-reducing conditions may be exploited for a determination as to whether antigen is present in a sample. More specifically, for example, as described herein, a polypeptide with an apparent molecular weight on SDS-PAGE of about 151,000 under reducing conditions exhibits a lower molecular weight of about 138,000 under non-reducing conditions.

Alternatively, the presence of antigen may be detected by immunological means. The means for detecting the presence of antigen may be in a direct or indirect test format. In a direct test format, that which is observed or measured is proportional to (i.e., directly reflective of) antigen present in a sample. Conversely, in an indirect test format, that which is observed or measured is inversely proportional to (i.e., indirectly reflective of) antigen present in a sample. Indirect formats include competitive and inhibition assay formats. As used herein, the term "antibody" includes both polyclonal and monoclonal antibodies; and may be an intact molecule, a fragment thereof, or a functional equivalent thereof; and may be genetically engineered. Examples of antibody fragments include $F(ab')_2$, Fab', Fab and Fv. Detection may be,

for example, by Western blot analysis utilizing antigen immobilized on nitrocellulose or Immobilon or similar matrix, in conjunction with specific antibodies to the antigen. Detection can also be achieved by immunoassay. In one embodiment, antigen is isolated from a sample and contacted with an appropriate detection antibody. Antigen may be isolated by capture on a solid support (e.g., heparin agarose) or with a "capture" antibody prior to or simultaneous with a "detection" antibody. In another embodiment, immunocomplexes are formed between an antibody and antigen, without prior purification of the antigen. Incubation of a sample with an antibody is under conditions and for a time sufficient to allow immunocomplexes to form. Detection of antigen by immunological means is also amenable to quantification where it is desired to determine the amount of antigen.

Detection of one or more immunocomplexes formed between antigen and an antibody specific for the antigen may be accomplished by a variety of known techniques, including radioimmunoassays (RIA) and enzyme linked immunosorbent assays (ELISA).

The immunoassays known in the art include the double monoclonal antibody sandwich immunoassay technique of David et al. (U.S. Patent 4,376,110); monoclonal-polyclonal antibody sandwich assays (Wide et al., in Kirkham and Hunter (eds.), *Radioimmunoassay Methods*, E. and S. Livingstone, Edinburgh, 1970); the "western blot" method of Gordon et al. (U.S. Patent 4,452,901); immunoprecipitation of labeled ligand (Brown et al., *J. Biol. Chem. 255*:4980-4983, 1980); enzyme-linked immunosorbant assays as described by, for example, Raines and Ross (*J. Biol. Chem. 257*:5154-5160, 1982); immunocytochemical techniques, including the use of fluorochromes (Brooks et al., *Clin. Exp. Immunol. 39*: 477, 1980); and neutralization of activity (Bowen-Pope et al., *Proc. Natl. Acad. Sci. USA 81*:2396-2400, 1984). In addition to the immunoassays described above, a number of other immunoassays are available, including those described in U.S. Patent Nos.: 3,817,827; 3,850,752; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; and 4,098,876.

For detection purposes, the antibodies may either be labeled or unlabeled. When unlabeled, the antibodies find use in agglutination assays. In

addition, unlabeled antibodies can be used in combination with other labeled antibodies (second antibodies) that are reactive with the antibody, such as antibodies specific for immunoglobulin. Alternatively, the antibodies can be directly labeled. Where they are labeled, the reporter group can include radioisotopes, fluorophores, enzymes, luminescers, or visible particles (e.g., colloidal gold and dye particles). These and other labels are well known in the art and are described, for example, in the following U.S. patents: 3,766,162; 3,791,932; 3,817,837; 3,996,345; and 4,233,402.

Typically in an ELISA assay the target antigen (for a competitive or inhibition assay format) or immobilized capture antibody is adsorbed to the surface of a microtiter well. Residual protein-binding sites on the surface are then blocked with an appropriate agent, such as bovine serum albumin (BSA), heat-inactivated normal goat serum (NGS), or BLOTTO (buffered solution of nonfat dry milk which also contains a preservative, salts, and an antifoaming agent). The well is then incubated with a sample suspected of containing antigen. The sample can be applied neat, or, more often, it can be diluted, usually in a buffered solution which contains a small amount (0.1%-5.0% by weight) of protein, such as BSA, NGS, or BLOTTO. After incubating for a sufficient length of time to allow specific binding to occur, the well is washed to remove unbound protein and then incubated with a detection antibody labeled with a reporter group, or an anti-immunoglobulin antibody labeled with a reporter group. The reporter group can be chosen from a variety of enzymes, including horseradish peroxidase, beta-galactosidase, alkaline phosphatase, and glucose oxidase. Sufficient time is allowed for specific binding to occur, the well is again washed to remove unbound conjugate, and the substrate for the enzyme is added. Color is allowed to develop and the optical density of the contents of the well is determined visually or instrumentally.

In one preferred embodiment of the present invention, a reporter group is bound to the detection antibody. The step of detecting an immunocomplex involves removing substantially any unbound antibody and then detecting the presence or absence of the reporter group.

In another preferred embodiment, a reporter group is bound to a second antibody capable of binding to the antibody specific for antigen. The step of detecting

an immunocomplex involves (a) removing substantially any unbound antibody, (b) adding the second antibody, (c) removing substantially any unbound second antibody and then (d) detecting the presence or absence of the reporter group. Where the antibody specific for the fragment is derived from a mouse, the second antibody is an anti-murine antibody.

In a third preferred embodiment for detecting an immunocomplex, a reporter group is bound to a molecule capable of binding to the immunocomplex. The step of detecting involves (a) adding the molecule, (b) removing substantially any unbound molecule, and then (c) detecting the presence or absence of the reporter group. An example of a molecule capable of binding to the immunocomplex is protein A.

It will be evident to one skilled in the art that a variety of methods for detecting the immunocomplex may be employed within the present invention. Reporter groups suitable for use in any of the methods include radioisotopes, fluorophores, enzymes, luminescers, and visible particles (e.g., colloidal gold and dye particles).

As disclosed herein, this antigen, which is associated with the presence of cancer, is bound by complement factor fragment C3b. Therefore, C3b may be used in assays (such as those described above) that utilize a capture molecule and a detection molecule for detecting antigen. For example, C3b may be immobilized on a solid support and used to capture antigen when contacted with a sample containing antigen. Another molecule which is specific for antigen, such as an antibody, may be used to detect any antigen bound to immobilized C3b. It may be desirable to wash the immobilized C3b, after introducing a sample suspected of containing antigen, prior to and/or subsequent to contacting with a detection molecule. Alternatively, this antigen possesses enzyme cofactor activity, e.g., whereby it acts as a cofactor for the digestion of C3b by Factor I of the complement system. Therefore, the presence of antigen may be determined by contacting a sample (suspected of containing antigen) with C3b and Factor I, and assaying for the digestion of C3b. In the presence of antigen and Factor I, the C3b α' fragment at a molecular weight of about 108,000 disappears with the concurrent appearance of fragments with molecular weights of 67,000 and 47,000. This digestion may be detected by a variety of ways, including SDS-PAGE.

Alternatively, rather than detecting the antigen itself, a nucleic acid molecule encoding the antigen can be detected. Such a nucleic acid molecule may be a deoxyribonucleic acid (DNA) or a ribonucleic acid (RNA). Generally, a nucleic acid molecule encoding for the antigen is detected by amplification of the nucleic acid. A variety of methods may be utilized in order to amplify a selected sequence, including, for example, RNA amplification (see Lizardi et al., *Bio/Technology 6*:1197-1202, 1988; Kramer et al., *Nature 339*:401-402, 1989; Lomeli et al., *Clinical Chem. 35*(9):1826-1831, 1989; U.S. Patent No. 4,786,600), and DNA amplification utilizing ligase chain reaction ("LCR") or polymerase chain reaction ("PCR") (see U.S. Patent Nos. 4,683,195, 4,683,202, and 4,800,159) (see also U.S. Patent Nos. 4,876,187 and 5,011,769, which describe an alternative detection/amplification system comprising the use of scissile linkages), or other nucleic acid amplification procedures that are well within the level of ordinary skill in the art. With respect to PCR, for example, the method may be modified as known in the art. Transcriptional enhancement of PCR may be accomplished by incorporation of bacteriophage T7 RNA polymerase promoter sequences in one of the primary oligonucleotides, and immunoenzymatic detection of the products from the enhanced emitter may be effected using anti-RNA:DNA antibodies (Blais, *Appl. Environ. Microbiol. 60*:348-352, 1994). PCR may also be used in combination with reverse dot-blot hybridization (Iida et al., *FEMS Microbiol. Lett. 114*:167-172, 1993). PCR products may be quantitatively analyzed by incorporation of dUTP (Duplàa et al., *Anal. Biochem. 212*:229-236, 1993), and samples may be filter sampled for PCR-gene probe detection (Bej et al., *Appl. Environ. Microbiol. 57*:3529-3534, 1991).

Primers for the amplification of a selected sequence should be selected from sequences that are highly specific to the antigen and form stable duplexes with the target sequence. The primers should also be non-complementary, especially at the 3' end, should not form dimers with themselves or other primers, and should not form secondary structures or duplexes with other regions of DNA. In general, primers (such as those described in greater detail below) of about 18 to 30 nucleotides are preferred, and can be easily synthesized using techniques well known in the art. PCR products,

and other nucleic acid amplification products, may be quantitated using techniques known in the art (Duplàa et al., *Anal. Biochem. 212*:229-236, 1993; Higuchi et al., *Bio/Technology 11*:1026-1030).

A preferred embodiment involves assaying for the presence of specific messenger RNA (mRNA) encoding the antigen. More specifically, for example, as described herein, a cell sample may be lysed and the mRNA isolated, amplified and examined for the presence of mRNA specific for the antigen. A variety of procedures may be used to detect the presence of antigen-specific mRNA. A particularly preferred method includes RT-PCR (Reverse Transcriptase based Polymerase Chain Reaction) amplification of mRNA.

Detecting the presence of antigen in a cell, tissue or sample has a variety of uses. For example, the present invention may be used for diagnostic purposes to screen warm-blooded animals, such as humans, for cancer (or a particular cancer depending upon the source of the particular cell, tissue or sample). Similarly, the present invention may be used to monitor warm-blooded animals. In particular, a preferred use is to follow patients who have been previously diagnosed and treated for cancer. Patients who are in remission (or may in fact be cured) can be monitored for the reappearance of cancer. It will be evident to those in the art that it may be desirable to use the present invention in conjunction with one or more other tests for cancer (or a particular cancer) to confirm positive or negative results obtained from use of the present invention.

The unexpected presence of a complement Factor H-related protein in cell culture supernatants from epithelial cancer cells (Example VI, Table 7), and the demonstration that its mRNA is produced by cancer cells (Example VI, Table 7), suggest that it plays a significant role in cancer biology. Data presented below (Example III.F) demonstrate that a biological activity of the antigen is to accelerate the complement Factor I-mediated degradation of C3b. The role of C3b *in vivo* is the assembly of the membrane attack complex (MAC) prior to lysis of an appropriate target. Because these proteins are members of the Alternative Complement Pathway, cell lysis may take place independent of the presence of circulating antibodies to any of

the cancer cell antigens. Although not wishing to be bound by theory, in view of the activity of the antigen described herein, its production by cancer cells may locally promote the degradation of C3b, thereby inhibiting the formation of the MAC and preventing tumor cell lysis by complement. Since the production of the antigen by tumor cells may afford a survival advantage, interrupting the production of the antigen or blocking its decay accelerator activity restores susceptibility of the tumor to complement-mediated cell lysis, thus offering a new approach to cancer therapy.

Irrespective of the exact function(s) of the complement Factor H-related protein in tumor biology, the present invention provides for the modulation of the antigen as a means for treating cancers. It will be evident to those of ordinary skill in the art that the antigen may be modulated in a variety of ways. A preferred method of interrupting the production of the antigen is by use of DNA, or PNA (peptide nucleic acid), constructs with base sequence complementary to the antigen's mRNA. Such an approach is generically termed antisense technology. Typically, the complement Factor H antisense DNA is inserted into an appropriate vector (virus) which delivers it to the tumor cells. Once inside the target cells, the antisense construct would specifically bind to mRNA coding for the complement Factor H-related protein, thereby preventing its translation. Primary among the other methods which could be used to interrupt production of the antigen would be the use of specific molecules which block the transcription of the specific gene or genes coding for the complement Factor H-related protein. Chemicals designed to block the ability of the tumor cell to produce antigen would preferably be delivered in the vicinity of the tumor, rather than systemically, since systemic introduction of such materials could decrease the normal production of complement Factor H by the liver, compromising the host's ability to regulate complement activity. In modulation of antigen production, it is desired to eliminate the production of all complement Factor H-related protein by tumor cells.

Another approach to antigen modulation is to use reagents to inhibit the activity of complement Factor H activity. Unlike inhibition of antigen production, the dosage used with these reagents should preferably result in an inhibition of 70%-95% of the Factor H activity. One family of such inhibitors—monoclonal antibodies, or

fragments which bind the antigen at a site which blocks its ability to degrade C3b—is presented as a representative example of modulation of antigen activity as an approach to cancer therapy (Example VII). In this example, certain antibodies which bind antigen are shown to accelerate the complement-mediated lysis of rabbit red blood cells and HL-60, a human tumor cell line. With these reagents, as with those described above, delivery should preferably be administered to the tumor site, rather than systemically. For the antibodies described above, reagent affinities should be at least about 10^6 liters/mole and doses should be within the range of about 0.01 μg/kg body weight to 10 mg/kg body weight. In addition, the preferred type of tumor to be treated in this manner would be distinctly separate from the circulatory system, since blood itself contains high concentrations of complement Factor H. An antibody may be replaced by, or supplemented with, any peptide or other organic molecule which specifically binds to complement Factor H-related protein and blocks its biological activity.

The above-described molecules (antibodies, peptides, organic compounds and antisense nucleic acids or peptide nucleic acids) are representative examples of agents that may modulate a tumor-associated human complement Factor H-related antigen or a nucleic acid molecule encoding the antigen, for use as a medicament to treat a tumor cell. Such agents may be used for the manufacture of a medicament for the treatment of a tumor cell. Further, such agents may be combined with a pharmaceutically acceptable carrier or diluent to from a composition. Additional components, such as traditional chemotherapeutic compounds, may be included with such an agent or a composition thereof.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

EXAMPLE I

DEVELOPMENT OF MONOCLONAL ANTIBODIES

A. Antigen

The antigen source for immunization was a pool of Heparin-Agarose fractionated urines from clinically diagnosed bladder cancer patients. (The purification method is described in detail in Example III.A.1. below.) Twenty-four hour urine samples were centrifuged in a Beckman centrifuge (Fullerton, CA), Model #J2-21, S/N 5539, using a JA-10 rotor at 6,000 rpm for 20 minutes. The clarified urine sample was then concentrated using an Amicon stirred cell, 76 mm, (cat# 5124) fitted with a YM30 membrane MWCO 30,000 dalton (Amicon, cat# 13742) or a Microgon hollow fiber concentrator, 50,000 MWCO (cat# M15S-260-01N) to approximately 100X concentration. The concentrated sample was diluted 1:1 with 25 mM Tris-HCl pH 7.4 and loaded onto a column of Heparin-Affigel (BioRad, Richmond, CA, cat# 153-6173), equilibrated in 25 mM Tris-HCl pH 7.4, at a flow rate of 2.0 mL/min. The sample was followed with equilibration buffer until the A_{280} elution profile returned to background. Bound material was eluted with a linear NaCl gradient from 0 to 250 mM NaCl in 25 mM Tris-HCl pH 7.4, followed by 50 mL of 250 mM NaCl, 25 mM Tris-HCl, pH 7.4, and finally 20 mL of a 10X PBS, pH 7.4, solution. Five mL fractions were collected and fractions from the trailing half of the elution peak were pooled. Pooled fractions were concentrated with an Amicon stirred cell, 43 mm (cat# 5122), fitted with a YM30 membrane, MWCO 30,000 dalton (cat# 13722). Fractions comprising the pooled antigen are shown below:

Pool I

Patient 1	fractions 13-31
Patient 1	fractions 14-30
Patient 2	fractions 11-19
Patient 3	fractions 11-24

Pool II (1.5 mg/mL)

Pool I	1 mL
Patient 2	fractions 11-19 1 mL
Patient 3	fractions 11-24 1 mL
Patient 4	fractions 11-20 1 mL

B. Immunizations

Five female BALB/c mice, of 8-10 weeks of age, were immunized intraperitoneally with 0.2 mL of a 1:1 emulsion of Pool II in Freund's Complete Adjuvant (Difco, Detroit, MI). Three weeks later, booster immunizations of 0.1 mL containing 10 μg of protein of an emulsion in incomplete Freund's Adjuvant was administered to the rear footpads and peritoneum. Ten days later each mouse was sampled for antibody response via retro-orbital bleeds and the sera were tested via an ELISA described below for titers. Mouse number 340 showed the highest titer and was chosen for fusion four days after boosting in the footpads and peritoneum with 15 μg of Pool II in phosphate buffered saline.

C. Fusion

Four days after the last immunization animal # 340 was sacrificed, the popliteal and inguinal lymph nodes and the spleen were collected and used for fusion. Fusion was carried out by a modification of the method of Fazekas De St. Groth and Scheidigger, *J. Immunol. M.* 35:1-21, 1980. The parent hybridoma line FO (ibid.), obtained from the ATCC, was used for fusion, at a ratio of one to five lymphocytes. PEG-DMSO (Sigma, St. Louis, MO) fusogen was used, and the cells plated out in Iscove's Modified Dulbecco's Medium (IMDM) with penicillin-streptomycin and hypoxanthine/thymidine (HT) supplement at a density of 2×10^4 cells/well with 2.58×10^3 peritoneal macrophages from unimmunized BALB/C mice added as feeders. The fusion was divided into two parts, in the first part forty-eight 96 well plates were seeded at the above density in media containing 1% fetal bovine serum (FBS). The second part consisted of 49 plates seeded at the same density in media containing 10% FBS. A total

of 97 plates, or 9,312 wells were used. The plates were incubated at 37°C in 7% CO_2 at 100% humidity. The next day 100 µl of selective media consisting of IMDM-HT with 2x methotrexate (8×10^{-7} M) and appropriate FBS concentration was added. The plates were returned to the incubator and not disturbed for six days. On day seven the plates were removed from the incubator and approximately 150 µl of media was removed via aspiration with a sterile eight place manifold. Complete IMDM with HT and appropriate FBS was added to each well using a Brinkman eight place pipette. The plates were returned to the incubator for another five to six days before screening. The fusion plates were examined each morning for wells showing growth levels suitable for screening, and were analyzed that day.

Within one week of the fusion, the plates containing the 1% FBS medium were clearly lagging in growth, and were therefore supplemented to 10% FBS. Thereafter, those wells selected from the plates initially plated in 1% FBS were designated as MOFI-followed by a number indicating the order of selection, those from the 10% FBS plates were designated with the MOFX prefix.

D. Post-Fusion Cell Culture

Wells selected via the screening assays were immediately transferred to 24 well plates containing 1 mL of complete IMDM containing 10% FBS. A sample of cells was also used to immediately re-clone the hybridomas by a serial limiting dilution procedure. This consisted of transferring a 10 µl sample of cells from the chosen well of the 96 well plate to the first well of a fresh 96 well plate previously filled with 100 µl of complete IMDM with 10% of a cloning supplement prepared from murine macrophages and thymocytes (Condimed, Boehringer-Mannheim Corp., Indianapolis, IN). Cells from the first well were serially diluted in the first column of wells by transferring 100 µl from the first well to the second, then from the second to the third, etc. The remaining 100 µl removed from the last well of the column is transferred back to the first well. The wells of the first column were then serially diluted across the plate by transfer of 50 µl of cell suspension using an 8 place pipette. Finally, 100 µl of cloning media was added to each well, and the plates incubated for approximately two weeks before subclones were ready for re-screening. Following growth in the 24 well

plates, the clones were transferred to six well plates with 5-6 mL of culture media, the plates were incubated until near confluent growth was observed. A sample of the cells were removed for storage in a cryogenic freezer in 5% DMSO in FBS, and the remaining cells were transferred to a T-75 flask with 10 mL media for producing spent media for further testing.

E. Stabilization of Subclones

Subclones were again subjected to testing via ELISA (described below) incorporating an additional urine from a patient diagnosed as TCC+. Typically all subclones of a given original-evaluated well showed similar binding patterns and levels. Those showing loss of antibody production in all subclones were discarded, while those displaying loss in any examined subclone were subjected to another subcloning. This was repeated until all subclones showed comparable levels of expression. Nomenclature for each level of subcloning consisted of appending to the clone designation a period followed by the number of the selected subclone.

F. Assays

The titer assay was carried out by coating Pool II (Example I.A., above) antigen adjusted to 4 μg/mL in 0.1 M carbonate buffer, pH 9.6, directly to polystyrene plates. Each well received 50 μl of coating solution and the plate was covered and incubated at 37°C for 2 hours, after which time it was washed twice with phosphate buffered saline (PBS) in a Denley strip well washer. The plate was blocked by the addition of 100 μl of a 1% gelatin hydrolysate, 2% sucrose solution in 50mM Tris-HCl, pH 7.5, at 37°C for 1 1/2 hours (all reagents from Sigma). Following blocking, the plate was again washed twice with PBS, then two-fold serial dilutions of mouse serum, starting at 1:100, into 10% normal horse serum in PBS, were added row-wise to the plate at 50 μl per well. The plate was incubated at 37°C for 1 hour, washed 4 times in PBS, and 50 μl of affinity purified goat anti-mouse IgG- horseradish peroxidase (HRP) conjugate (Tago, Burlingame, CA) diluted 1:5000 in 10% horse serum in PBS added to each well. This was allowed to incubate for 1 hour at 37°C. The plate was washed with PBS 4 times, and 50 μl of substrate (K-Blue, ELISA Technologies, Lexington, KY) was

added and the plate allowed to develop for 10 minutes at room temperature before stopping the reaction via the addition of 100 µl of 2M phosphoric acid solution in water (Sigma). The optical density of the wells were read at 450 and at 410 nm in a BioTek EL311 plate reader. Readings which were off scale at 450 nm were calculated from the corresponding reading at 410 nm by the method of Madersbacher and Berger, *J. Immunol. M. 138*:121-124, 1991.

The fusion was screened for antibody production by use of the following fusion screen. Antibody binding was tested with: (a) two clinically diagnosed patient urines, stages T2III and T3III, (diluted 1:80), (b) two pools of normal human urines (diluted 1:15), (c) human type IV collagen (diluted to 4 µg/mL), all dilutions in 25mM Tris-HCl, and (d) pooled human red blood cells (Gamma Biologicals, Houston, TX) diluted into PBS and coated onto poly-lysine coated plates. All plates were blocked by washing with PBS with 0.1% Tween-20, and by the dilution of the media samples 1:5 into complete IMDM containing 10% FBS. Supernatant fluid (70 µL) of the wells chosen for screening were transferred to a well of a 96 well plate. To each well, 280 µl of diluent was added, and 50 µl was distributed to the test plate wells. The remaining steps of the assay were as for the titer assay, with the exception that the conjugate used was human serum adsorbed goat anti-mouse IgG-HRP conjugate (Kirkegaard and Perry Labs (KPL), Gaithersburg, MD) diluted 1:5000 in 10% normal goat serum in PBS for all except the RBC plates. For the latter, an alkaline phosphatase conjugate of a similar antibody was used (KPL, Gaithersburg, MD) followed by use of PNPP (p-nitrophenyl phosphate) substrate. Controls were used for each assay, negative control was fresh IMDM with 10% FBS, positive controls were monoclonal anti-human collagen (Sigma C1926), and monoclonal anti-hIgA (A1.1.2.4, Bard Diagnostic Sciences, Inc., Redmond, WA), both of which showed high binding to all test antigens except the red blood cells. Criteria for selection were high binding to cancer urine plates (OD>1), low binding to normal urines and other test antigens (OD<0.5). Others which showed high antibody levels in different patterns with respect to the test antigens were also selected for potential research uses.

Subclones were screened by several assays. First, the fusion assay was again used then, following expansion in culture of selected subclones, an abbreviated ELISA was employed using normal urine pool I and the two advanced stage urines used in the fusion assay. The testing was carried out at dilutions of 1:10 and 1:100 for the early subclones, and an additional dilution of 1:1000 for the later subclones. In several of the subclone assays the addition of urine from a patient with a lower grade cancer was included.

From the 9,312 wells plated in the fusion, a total of 880 wells showing growth were screened, with a total of 94 X series and 24 I series clones selected for further work. Analysis of the fusion via Poisson distribution, suggested that there was a 4.6% probability that any well showing growth contained 2 or more clones, or 5 to 6 of the total clones being multiclonal. Of the 118 clones selected, 37-X and 8-I series were eventually lost due to instability or lack of growth without feeder cells.

A total of 32 subclones were selected based on selectivity of antibody binding to cancer positive urines versus the normal urines and on retention of assay OD with dilution of culture supernatant to select for high affinity and good production level. Samples of spent culture media from the following clones were evaluated for their potential utility in a clinical assay for the antigen described in Example III: I-7.3, I-8.2, I-10.2, I-11.1, I-12.2, I-17.3, X-4.1, X-13.1, X-13.2, X-22.2, X-28.1, X-44.1, X-48.1, X-49.1. X-49.2, X-50.3, X-52.1, X-53.2, X-55.1, X-56.3, X-59.1, X-60.2, X-61.2, X-62.1, X-63.2, X-64.3, X-67.2, X-69.1, X-70.2, X-84.2, and X-87.2. A preferred monoclonal antibody pair for assays is X-13.2 (conjugate MAb) and X-52.1 (capture MAb).

EXAMPLE II

DEVELOPMENT OF GOAT POLYCLONAL ANTIBODIES

A. Goat Immunization

1. Antigen

Heparin-Agarose chromatography (Example I.A., above) fractions from three TCC-positive patients were pooled and dialysed against phosphate buffered saline (PBS). Protein concentration was determined to be 2 mg/mL. Thimerosal was added to a final concentration of 0.02%, and 0.25 mL aliquots were frozen until use. Table 1 is a listing of the amounts and references of the antigens comprising Pool I.

Table 1

Antigen Pool I

Patient ID	Date of Sample	Fractions	Protein Conc.	Volume Used
1	3/29/94	13-31*	–	–
1	3/30/94	14-30*	3.2 mg/mL*	0.5 mL
2	6/6/94	11-19	0.91 mg/mL	1.5 mL
3	6/8/94	11-24	3.6 mg/mL	0.7 mL

* both sets of fractions were combined before protein determination.

2. Immunization

For immunization, a vial of antigen was thawed and mixed. An aliquot of 0.125 mL of antigen was mixed into 0.75 mL of PBS, and drawn into a 5 mL glass syringe. This syringe was attached to another such syringe containing 1 mL of Difco Freund's adjuvant, via a double-hub emulsifying needle. The first immunization was with Freund's Complete adjuvant, all others were with Freund's incomplete adjuvant. The emulsion was formed by alternately forcing the total mixture from one syringe to the other. The stability of the emulsion was tested by removing one syringe from the needle and dipping the end into a beaker of tap water. If a small amount of emulsion expressed into the water did not immediately spread over the surface, the emulsion was

deemed stable. The entire amount of emulsion was collected into one syringe, which was capped and stored on ice until used. Total protein in the inoculum was 0.25 mg.

Goats (R & R Rabbitry, Inc., Stanwood, WA) were 5 1/2 months of age and weighed approximately 34 kg. when the first immunization was administered. The second and third immunizations were given thirty and sixty days later.

B. Antibody Analysis

Serum samples were taken pre-immunization and two weeks after the second and third immunizations and were analyzed via ELISA using the antigen coated onto microplates. The assay was similar to the ELISA used for the mouse serum titer with the exception that antigen Pool I and rabbit anti-goat IgG-HRP were used and the dilution range employed was from 6×10^3 to 1.861×10^6.

The results of the assay were the following:

1. Pre-immunization sample showed no antibody titer as expected.

2. Samples after the second and third immunizations showed a maximal OD at about 3×10^3 dilution, a half maximal signal at about 8×10^4 and background at 1×10^6. The signal at a dilution of 1×10^5 was 1.4.

3. In another experiment, cross reactions were tested and rated on a scale of 0 to 4 at a serum dilution of 1×10^5, and were as follows in Table 2, with 4 being highest OD:

Table 2

human Collagen Type IV	0
human Fibronectin	1
human Laminin	0
human Fibrinogen	1
Bovine submaxillary mucin	0
human red blood cells	0
Pool I	4

(rabbit anti-goat IgG-alkaline phosphatase was substituted for the rabbit anti-goat IgG-HRP).

EXAMPLE III

PURIFICATION AND CHARACTERIZATION OF ANTIGEN

A. Purification

1. Heparin-Agarose Chromatography of Urine

Twenty-four hour urine samples were centrifuged in a Beckman centrifuge, Model #J2-21, S/N 5539, using a JA-10 rotor at 6,000 rpm for 20 minutes. The clarified urine sample was then concentrated using an Amicon stirred cell, 76 mm, (cat# 5124) fitted with a YM30 membrane MWCO 30,000 daltons (Amicon, cat# 13742) or a Microgon hollow fiber concentrator, 50,000 MWCO (cat# M15S-260-01N) to approximately 100X concentration. The concentrated sample was diluted 1:1 with 25 mM Tris-HCl, pH 7.4, and loaded onto a column of Heparin-Affigel (BioRad, cat# 153-6173), equilibrated in 25 mM Tris-HCl pH 7.4, at a flow rate of 2.0 mL/min. The sample was followed with equilibration buffer until the A_{280} elution profile returned to background. Bound material was eluted with a 100 mL, linear NaCl gradient from 0 to 250 mM NaCl in 25 mM Tris-HCl, pH 7.4, followed by 50 mL of 250 mM NaCl, 25 mM Tris-HCl, pH 7.4, and finally 20 mL of a 10X PBS, pH 7.4, solution. Five mL fractions were collected and fractions from the trailing half of the elution peak were pooled. Pooled fractions were concentrated with an Amicon stirred cell, 43 mm (cat# 5122), fitted with a YM30 membrane, MWCO 30,000 daltons (cat# 13722).

2. Protein A Chromatography of 24 Hour Urine

Protein A Chromatography was performed on a 24 hour urine from a TCC+ patient to determine whether this tumor antigen could be part of an immune complex. The urine (6 mL) was diluted to 12 mL with the addition of 6 mL of 20 mM sodium phosphate, pH 7.4. The diluted urine (7.3 mL) was loaded on a 1.0 mL Protein A cartridge (BioRad, Richmond, CA, cat# 732-0093) equilibrated in 20 mM sodium phosphate, pH 7.4, at 0.5 mL/min. The flow through volume plus 5.0 mL of wash buffer was collected and labeled as "flow through" (total volume = 12.3 mL). Bound

material was eluted with 100 mM citrate buffer, pH 3.0, and neutralized immediately with the addition of 100 µl of a 1.0 M Tris-HCl, pH 8.0, to each 3 mL fraction. Eluted antigen was pooled (~ 6 mL) and the sample load, flow through, and eluted pool, at dilutions of 1:20 to 1:2560, were tested in the double monoclonal microtiter plate assay described in detail below (Example IV.B.). Approximately 97.5% of the activity loaded was contained within the flow through peak. The 2-3% activity in the eluted pool was probably due to incomplete washing. Thus, this antigen is not part of an immune complex involving IgG, and the use of immobilized Protein A would not be effective in extracting the antigen from specimens.

3. MAb 13.2 and MAb 52.1 Affinity Chromatography of 24 Hour Urine

Aliquots of 24-four hour urines were diluted 1:1 with 25 mM Tris-HCl, 250 mM NaCl, pH 7.4, and loaded onto 5 mL MAb affinity columns (BioRad A10 gel) prepared with MAb X-13.2 or MAb X-52.1 (Example I.F.). To serve as a control for urine materials binding non-specifically to IgG, an A10 control column was prepared using Protein A-purified, normal mouse serum. Samples were loaded at 0.5 mL/min. The sample was eluted with 25 mM Tris-HCl, 250 mM NaCl, pH 7.4, until the OD280 baseline was reached. The bound material was then eluted with 100 mM glycine-HCl, pH 3.0. The eluted fractions, 5 mL each, were collected in tubes containing 100 µl of 1.0 M Tris-HCl, pH 8.0. Purity was assessed by SDS-PAGE using Novex 8-16% and/or 4-12% polyacrylamide gels under reducing and non reducing conditions, along with Novex (San Diego, CA) Mark XII molecular weight standards (6 to 200 kD). The gels were stained with Coomassie Blue R250 followed by silver staining and scanned using a BioRad GS7000 densitometer. Molecular weights of individual bands are estimated based on the Rf values of the molecular weight standards (Example III.C.).

B. Native Molecular Weight Estimation of the Antigen by Gel Filtration

A gel filtration column was prepared with Pharmacia Sephacryl S-300 (Pharmacia, Piscataway, NJ, Cat# 17-0599-01). Briefly, deionized water is added to S-300 gel to form a 50% slurry and added to a 1.0 L vacuum flask. The slurry was

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2. Results

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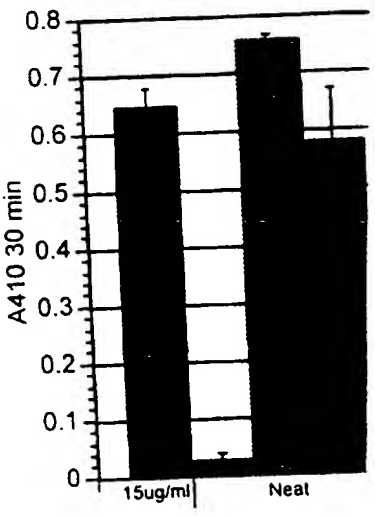

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bottom of the gel. The gel's protein bands were then transferred to PVDF and stained with Coomassie Blue R250.

Rf values were calculated for the Mark XII individual molecular weight standards by dividing the distance the band moved through the resolving gel by the distance of the sample front from the top of the resolving gel. A linear standard curve was established by plotting Rf values versus log MW for each MW standard. Sample bands' molecular weights were estimated by calculating their Rf values and entering these values (y_i) in the standard curve equation.

Immobilized MAb X-52.1 bound approximately 10 components with apparent molecular weights 151, 130, 87, 77, 60, 49, 39, 29, 25, and 10 kD under reducing conditions (i.e., in the presence of DTT). Only bands at 151, 130, and 39 kD appeared to be specific for the MAb X-52.1 in that the other proteins also bound to immobilized non-specific mouse IgG. Of these bands, that corresponding to a molecular weight of 151 kD is typically the most intensely staining. In contrast, immobilized MAb X-13.2 affinity purified fractions were generally cleaner than those obtained with immobilized MAb X-52.1, containing predominant bands at 151, 130, and 39 kD with only very minor contaminant bands at 77, 60, and 25 kD. Under non-reducing conditions, the monoclonal antibody-specific bands exhibited apparent molecular weights of approximately 138 kD, 121 kD, and 40 kD, with the 138 kD band being typically the most intense. The shift in apparent molecular weights of the dominant band from 138 kD to 151 kD upon reduction and the 121 kD band to 130 kD upon reduction could be due to the presence of a large number of intra-chain disulfide bonds in these molecules. This characteristic electrophoretic behavior formed the basis for the antigen assay described in Example IV.F.

D. Western Blot Analysis of Partially Purified Antigen Preparations

The urine samples purified on Heparin-Agarose (Example III.A.1.) were diluted 1:2 with SDS-PAGE 2X Sample Buffer (Novex, cat# LC 2676) in the presence dithiothreitol and heated at 100°C in a boiling water bath for 5 minutes, then allowed to cool to room temperature. The sample preparations were loaded onto an 8-16% acrylamide, 1.0 mm thick, 10-well, discontinuous Novex SDS-PAGE gel (Novex, San

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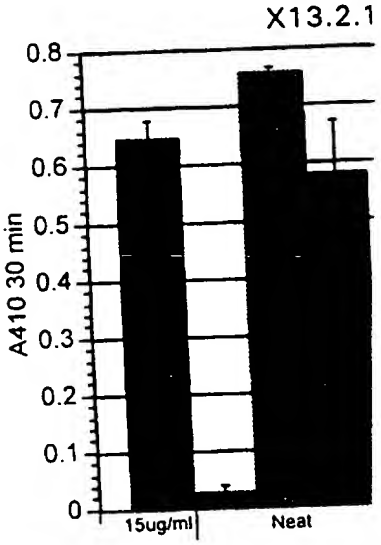

preparation was loaded onto an 8% acrylamide, 1.0 mm thick, 2-well, discontinuous Novex SDS-PAGE gel (Novex, cat# EC6012) and electrophoresed at 125 V constant for 190 V-h using a Novex electrophoresis chamber (Novex, cat# EI9001) and a BioRad Power Unit 500V (cat# 165-4710). BioRad SDS-PAGE Molecular Weight Standards (cat# 161-0317) were loaded into a reference well.

The gel was removed and placed in a container of 10 mM CAPSO buffer, pH 9.0, containing 0.05% SDS on a rocker platform while the gel transfer sandwich was prepared. The SDS-PAGE bands were transferred to PVDF membrane (Novex, cat# LC2002) using a Novex Transfer apparatus (Novex, cat# EI9051) and BioRad 500 power supply at 125 mA constant for 60 minutes. The PVDF membrane was removed and rinsed with deionized water and stained in a 0.1% Coomassie Blue R-250 in 20% methanol protein staining solution for approximately 10 minutes. The stained PVDF was then destained with several changes of 30% methanol until the background stain was minimal, and was followed by extensive washing in deionized water. The PVDF membrane was then allowed to dry at room temperature on a paper towel. The stained bands of interest were excised with a clean razor blade and placed in capped tubes. The samples were carried to the University of Washington (Seattle, WA) for sequencing by Edman degradation. The principal amino acid sequence thus obtained was: E D C N ? L P P R ? N T (SEQ ID NO:1), where the symbol "?" indicates a residue which could not be identified.

2. Trypsin Digestion: Internal Amino Acid Sequence

A small amount (50-100 µL) of immobilized trypsin (Pierce Chemical Co., Rockford, IL) was added to a 600 µL Eppendorf tube along with an equal volume of PBS. After gentle mixing, the slurry was spun down at 10K rpm for about 30 seconds. The supernatant solution was pipetted off and two times the slurry volume of PBS was added, mixed and the spin repeated. This wash step was repeated twice more and the slurry brought back to the original volume with PBS.

A known quantity of antigen was added to a clean 600 µL Eppendorf tube and PBS added to bring the concentration to 0.5 mg/mL. Immobilized trypsin was

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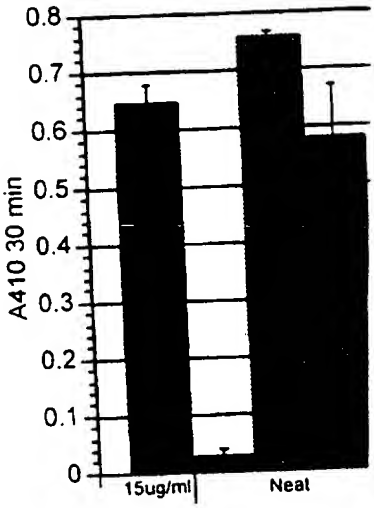

Table 3

Sequences of Urine Antigen Tryptic Fragments

Sample	[1] Fragment (~MW)	Sequence	SEQ ID
1	128 kD sequence starts at CFH AA 324	GPYFPVAVGKYY?(Y)Y?D - [RPYFPVAVGKYYS Y YCD]	NO:2 NO:12
2	79 kD sequence starts at CFH AA 324	RPYFPVAVGKYYS?Y?DE?F???S [RPYFPVAVGKYYSYYCDEHFETPS]	NO:3 NO:13
3	46 kD sequence starts at CFH AA 868	SSQESYAHGTK [SSQESYAHGTK]	NO:4 NO:4
4	37 kD sequence starts at CFH AA 1	EDCNELPP?RNTEIL?GSW-D [EDCNELPPRRNTEILTGSWSD]	NO:5 NO:14
5	66 kD sequence starts at CFH AA 324	RPYFPVVAVGKYYSYY?DEHFE?P [RPYFP-VAVGKYYSYYCDEHFETP]	NO:6 NO:15
6	33 kD sequence starts at CFH AA 40	[2] SLGNVIMV?RKGEWVALNPLRK [SLGNVIMVCRKGEWVALNPLRK]	NO:7 NO:16
	sequence starts at CFH AA 324	[3] RPYFPVAVGKY [RPYFPVAVGKY]	NO:8 NO:8

[1] Amino acid residue numbers refer to the mature CFH molecule
[2] Major protein sequence
[3] Minor protein sequence

The similarity of the partial amino acid sequences of the antigen with those of the reported sequence for human complement Factor H (shown in brackets) demonstrates that the antigen detected is a member of a complement Factor H-related family of proteins as disclosed herein.

F. C3b - Decay Accelerator Activity of the Antigen

C3b was prepared from C3 (Sigma Chemical Co., St. Louis, MO) by trypsin digestion, using immobilized trypsin (Pierce Chemical Co., Rockford IL), and an enzyme to substrate (E/S) ratio of 1:25 at room temperature for fifteen minutes. The digest was spun at 10k rpm for about 30 seconds to pellet the enzyme, and the supernate removed. The supernate was checked for the presence of C3b by SDS-PAGE under reducing conditions.

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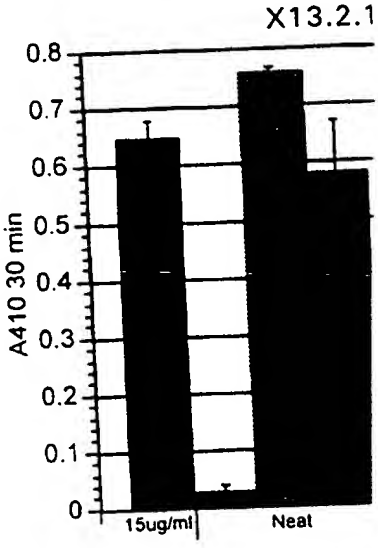

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EXAMPLE IV

ASSAYS FOR THE ANTIGEN

Given the characteristics of the antigen as described above and given the disclosure herein for generating and selecting antibodies and the development of certain assays described herein to detect the antigen, a number of additional assay formats beyond those described herein for this antigen may be readily developed by those of ordinary skill in the art. Suitable assay formats include competitive formats, sandwich formats (Examples IV.A., IV.B. and IV.C), assays based on the biological or chemical properties of the antigen (Example IV.D. and IV.E.), assays based on the simultaneous binding of the antigen to a specific macromolecule (e.g., C3b) and to a monoclonal antibody (Example IV.D.), assays based on the appearance of a band of appropriate size in partially-purified specimens (Example IV.F.), and RT-PCR (Example IV.G.). A preferred format involves sandwich immunoassays and the most preferred employs a monoclonal antibody immobilized on a solid surface and a second monoclonal antibody, which recognizes an epitope distinct from that of the first, conjugated to a detection agent. That detection agent could be an enzyme (Example IV.B.), colloidal gold (Example IV.C.), or any of a number of other such agents known to those of ordinary skill in the art. These include fluorescent molecules, radioisotopes, and biotin (which would subsequently bind to avidin or strepavidin-labeled detecting agent).

A. Identifying Potential Antibody Pairs

Definitions for the section:

Indirect Assay Format:	Antigen coated on plate; reaction with MAb; signal generation by Goat Anti-mouse conjugated to alkaline phosphatase (GAM-AP).
Direct Assay Format:	Antigen coated on plate; reaction with and signal generation by specific MAb-AP.
Sandwich Assay Format:	As usual

wash with Tris-buffered saline (T
plates were blocked with 100 µl p
37°C and washed four times. An
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washing, the plates were incubated with AP-conjugated antibodies from specific clones; (3) following a final wash, the plates were incubated with pNPP; and, finally, (4) the reactions were stopped and measured as above. Based on the results obtained from seven conjugates tested on a small number of urine samples in this manner, all seven were selected for further study in the sandwich format of the ELISA.

Thirteen monoclonal antibodies (Example I.F.) and one goat polyclonal preparation (Example II) were tested as capture antibodies in combination with the seven alkaline phosphatase conjugates in the sandwich ELISA format as follows: (1) individual capture antibodies were adsorbed on microtiter plates; (2) following washing, diluted urine samples were added to the wells and incubated to allow binding of the antigen to the antibody; (3) following another wash, single conjugates (as described in B. above) were added to individual wells and incubated to allow binding to the antibody-bound antigen, if present; (4) following a final wash, the plates were incubated with pNPP; and, finally, (5) the reactions were stopped and measured as above. A total of 107 potential antibody pairs were first tested against one normal and seven TCC-positive urine samples. From these, a selection of 33 pairs were chosen to be tested against an expanded series of urines from 31 patients and one normal individual. From the results of this testing, seven antibody pairs were selected for further testing against a much expanded selection of 120 patient urine samples, but including also 20 samples from normal individuals. From this extensive testing of these seven pairs, a single monoclonal antibody pair (X52.1/X13.2-AP) was selected as the most preferred on the basis of (1) its positive response with the greatest number of samples from TCC-positive patients, (2) its negative response with the greatest number of samples from non-TCC-positive patients, and (3) low nonspecific reaction with urine samples from normal, non-diseased individuals. In addition, an alternative antibody pair was selected (X52.1/X62.1-AP).

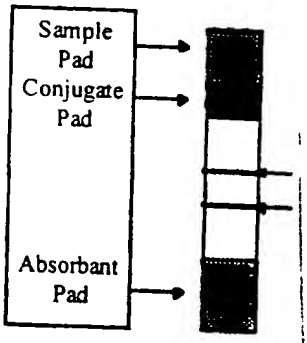
B. Sandwich ELISA

The sandwich ELISA, utilizing the most preferred pair as selected above, was further optimized with respect to the following items: (1) coating level of capture antibody; (2) concentration of conjugate; (3) enzyme-to-antibody ratio in the conjugate;

strip. A goat anti-mouse antibody (Chem
approximately 3/8 in. from the other edg
dried overnight under reduced pressure a
strips.

The 10.5 in. strips of coat
were then assembled onto plastic cards (p
sided tape (below). The membrane was fi
The conjugate pad was then placed so as to
(near the x52.1 immobilized antibody) of t
strip (Whatman, 10.5 x 0.75 in.) was placed
goat anti-mouse immobilized antibody) of t
cotton paper strip was placed in overlappin
the sample. The assembled cards were cu
placed into plastic housings which provided
viewing window in the nitrocellulose region

Configuration of lateral flow assay co



Positive

1. Results

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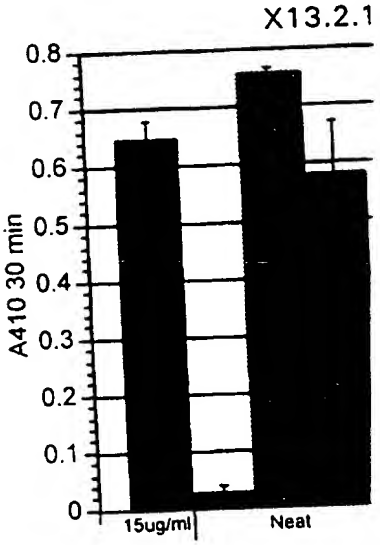


plate was performed at 37°C for 60 minutes. Following washing, 200 µl of working dilution of conjugate was added to the aspirated well. The covered plate was again incubated for 60 minutes at 37°C. Following a final wash, 200 µl of pNPP substrate was pipetted into each well, and the covered plate was incubated at 37°C for 30 minutes. After pipetting, 50 µl of stop solution into each well, the reaction mixtures in each well were measured at 410 nm.

4. Typical Results

Eighty seven urine samples were assayed by the ELISA using the format described above. These samples included 23 clinical specimens taken from patients diagnosed as currently having transitional cell carcinoma (TCC) and 64 others. The results are tabulated below in Table 4. Sensitivity is reported as the percentage of specimens from TCC-positive patients that correctly produce a positive result in the assay. Specificity is reported as the percentage of urines from individuals without TCC that correctly produce a negative result in the assay.

Table 4

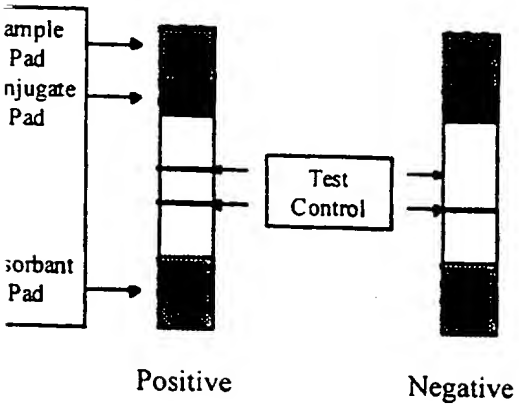
	Number of Specimens	Percentage of Total
Sensitivity	23	48%
Specificity		
Healthy	25	88%
Non-GU Disease	15	87%
GU Malignancy	3	100%
Other GU Disease	10	70%
Chronic Inflammation (Urinary Tract)	11	27%

A graphical representation of the data for each specimen, expressed as Units of analyte/mL, is given below. (Note that the categories correspond to those specified in the above table and that GU=Genitourinary.)

e antibody (Chemicon, Temecula, CA) at 2 mg/ml was sprayed om the other edge of the membrane. The membrane was then educed pressure at ambient temperature and cut into 10.5 in.

in. strips of coated glass fiber mesh and sprayed membrane to plastic cards (polypropylene, 10.5 x 2.25 in.) using double- membrane was first placed near the center of the plastic card. hen placed so as to overlap the membrane on the proximal side ized antibody) of the membrane and an absorbent cotton paper).75 in.) was placed so as to overlap on the distal side (near the lized antibody) of the membrane. Finally, a second absorbent laced in overlapping contact with the conjugate pad to accept bled cards were cut crosswise and the resulting small strips igs which provided a well for containment of the sample and a trocellulose region for reading the results.

lateral flow assay components:



ys were carried out by placing 250 µl of patient urine in the inutes, the results were read. A positive result will show a t zone (zone of immobilized X-52.1) and a pink-purple line in

wash with Tris-buffered sali plates were blocked with 10(37°C and washed four times 0.15M $MgCl_2$, 0.15M $ZnCl_2$, 37°C. The plates were wasl alkaline phosphatase,) was a for 30 minutes. After four v St. Louis, MO) at 1 mg/ml incubated for 30 minutes at 3 solution (0.1 M EDTA, pH reader.

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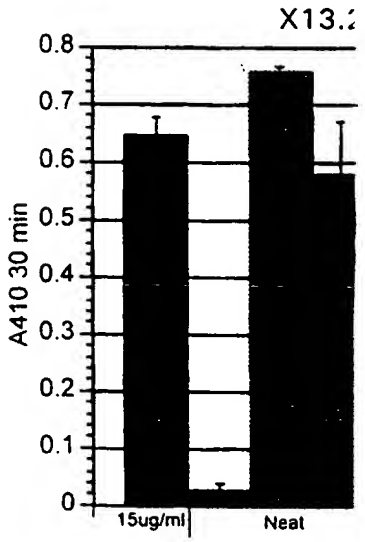

stage and/or of low grade. With respect to those patients identified as having chronic inflammation. several have a history of TCC.

C. Rapid Assay

Monoclonal antibodies specific for the antigen (Example I.F.) were utilized in a lateral flow format to produce a qualitative assay for bladder cancer using urine as the specimen. The lateral flow format consisted of a colloidal gold antibody conjugate and an immobilized capture antibody on a nitrocellulose membrane. Upon interaction of the urine sample with the colloidal gold conjugate, the antigen in the urine sample formed an antigen-antibody conjugate complex. This complex migrated by capillary flow through the membrane and contacted the immobilized anti-antigen capture antibody (test zone). The capture antibody bound the antigen-antibody conjugate complex, forming a visually detectable colored signal in the test zone. Material not bound by the capture antibody continued to migrate through the membrane and contact an immobilized goat anti-mouse antibody (control zone) which bound the colloidal gold conjugate regardless of the presence of antigen, forming a visually detectable signal in the control zone.

Purified monoclonal antibody X-13.2 was conjugated to colloidal gold according to Frens (Frens, G., *Nature, Phys. Sci. 241*:20-22, 1973). Briefly, the gold sol was prepared by reduction of tetrachloroauric acid by trisodium citrate. The solution was boiled until a color change was observed. MAb X13.2 was adsorbed to the gold sol at 0.3 mg/ml for 5 minutes at pH 9. The conjugate was blocked with 0.5% BSA and washed twice with the conjugation buffer. The washed conjugate was then diluted 7-fold into 2% BSA with 50mM Tris, pH 9, and 0.05% NaN_3. The washed conjugate was used to saturate strips (10.5 x 0.25 in.) of glass fiber mesh (Lydall, Hamptonville, NC). These conjugate strips were then dried overnight under reduced pressure at ambient temperature.

An airbrush sprayer was used to immobilize the capture and control antibodies on the membrane. Purified monoclonal antibody X-52.1 at 2 mg/ml was sprayed as a line onto a section of nitrocellulose membrane (8 μm pore size, 50m x 1 inch, Whatman, Fairfield, NJ) approximately 3/8 in. from one edge of the membrane

antibody (Chemicon, Temecula, CA) at 2 mg/ml was sprayed
n the other edge of the membrane. The membrane was then
luced pressure at ambient temperature and cut into 10.5 in.

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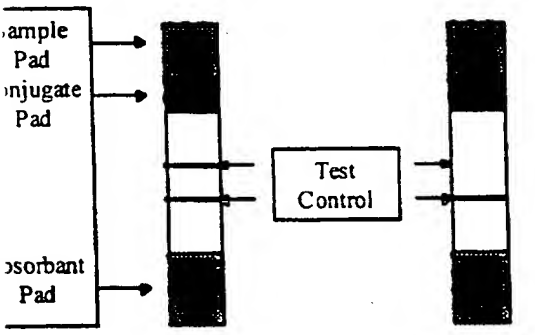

Positive Negative

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minutes, the results were read. A positive result will show a
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plates were blocked with 100 µ
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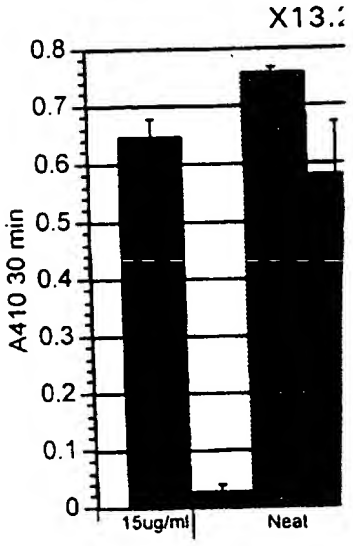
St. Louis, MO) at 1 mg/ml
incubated for 30 minutes at 3
solution (0.1 M EDTA, pH 9
reader.

2. Results

This assay for
and normal urines, yielding a



the control zone (zone of immobilized goat anti-mouse). A negative result will show no line in the test zone and a pink-purple line in the control zone. The absence of a line in the control zone indicates that the reagents in the test did not function properly and this test is invalid. Twenty three TCC-positive clinical specimens and 64 other urine specimens were assayed in the lateral flow assay. The results are given in Table 5 below. Sensitivity is reported as the percentage of TCC-positive specimens that correctly produced a positive result in the lateral flow assay. Specificity is reported as the percentage of TCC-negative urines that correctly produced a negative result in the lateral flow assay.

Table 5

Sensitivity (23)	65%
Specificity	
Healthy (25)	92%
Non-GU Disease (30)	57%
GU Malignancy (3)	100%
Other GU Disease (6)	83%

GU = Genitourinary

From Table 5 it is clear that the bladder cancer lateral flow assay detects a large percentage of the TCC-positive specimens tested and distinguishes bladder cancer (TCC) from other normal and disease states.

D. C3b-MAb ELISA

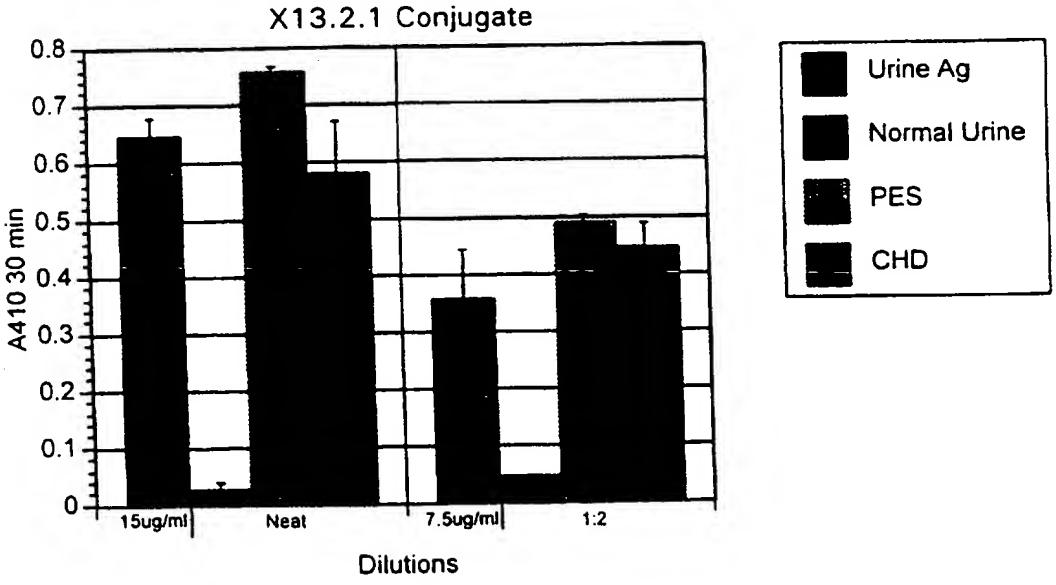
1. Method

Immulon 4 (Dynatech, Chantilly, VA) microtiter strip wells were coated with 50 µl per well of 5 µg/ml trypsin treated C3 (converts C3 to C3b; see Example III.F. above for C3 source and method of activation) in 50 mM carbonate buffer, pH 9.6, either overnight at 4°C or for two hours at 37°C. A control plate was coated with 50 µl per well of 2% BSA in PBS for two hours at 37°C. After a single

wash with Tris-buffered saline (TBS) containing 0.1% Tween -20 (wash buffer), the plates were blocked with 100 µl per well of a 2% BSA solution in PBS for two hours at 37°C and washed four times. Antigen, diluted in assay diluent (1% BSA in TBS with 0.15M $MgCl_2$, 0.15M $ZnCl_2$), was added at 50 µl per well and incubated for one hour at 37°C. The plates were washed four times and then the detection antibody (x13.2.1.1-alkaline phosphatase,) was applied at 0.25 µg/ml, 50 µl per well, and incubated at 37°C for 30 minutes. After four washes, 50 µl per well of p-nitrophenyl phosphate (Sigma, St. Louis, MO) at 1 mg/ml in 1M diethanolamine (DEA) was added and the plate incubated for 30 minutes at 37°C. The reaction was stopped with 25 µl per well of stop solution (0.1 M EDTA, pH 9.8) and the plate read at 405 nm on a Dynatech MR7000 reader.

2. Results

This assay format discriminated between TCC^+ urines (PES and CHD) and normal urines, yielding a positive signal for the TCC^+ urines.



E. C3b - Decay Accelerator Activity Assay

The assay described above in the C3b decay accelerator activity section (Example III.F.) discriminated three patient urines (all TCC^+) from a normal urine pool. Therefore, it may be used as an assay to indicate bladder cancer in patients.

F. SDS-PAGE Assay

The antigen partially purified by heparin agarose chromatography (Example III.A.) may also be detected by SDS-PAGE under reducing and non-reducing conditions, since it displayed a characteristic apparent molecular weight shift upon reduction (Example III.D.). As described above, the reduced antigen exhibited an apparent molecular weight (as estimated by SDS-PAGE) of ~151 kD as compared to ~138 kD under non-reducing conditions, presumably due to numerous disulfide bridges. The antigen containing peak from heparin chromatography was diluted in 2X SDS-PAGE sample buffer with and without dithiothreitol (DTT) at 5%. The samples were heated for 5 minutes in a boiling water bath and then allowed to cool at room temperature. Aliquots (2 µL) were loaded onto an 8 well, 7.5% acrylamide Pharmacia PhastGel (cat# 17-0622-01) and run on the Pharmacia PhastSystem (cat# 18-1018-23) according to manufacturer's protocol. Reduced and non-reduced samples were run on the same gel and were separated by molecular weight standards and an empty lane loaded with non-reducing 1X sample buffer. The gels were stained with Coomassie Blue R250 0.1% in 40% methanol and 10% acetic acid and then destained with 40% methanol with 10% acetic acid. Characteristic bands were seen on specimens with elevated antigen levels as detected by the ELISA (Example IV.B.).

G. RT-PCR Assay

1. Cell Lines

Several cell lines, particularly cell lines HTB-5 and HTB-9, which are derived from Transitional Cell Carcinoma (TCC) of the bladder and HeLaS3, which is derived from adenocarcinoma of the cervix (all from American Type Culture Collection, Rockville, MD), were tested to determine whether they produce mRNA coding for the antigen. Although the method selected for cell line analysis was RT-

PCR (Reverse Transcriptase based Polymerase Chain Reaction amplification of messenger RNA, mRNA), a variety of procedures used to detect the presence of specific RNA can be used. Controls were performed using PCR target materials (the PAW109 sequence) provided with commercial PCR kits, and its primers DM152 and DM151. Hybridoma cell line X-44.1 or normal human epithelial keratinocytes (Clonetics Corp., San Diego, CA) were chosen as the irrelevant target (Negative controls).

2. Preparation of mRNA

Preparation of mRNA was facilitated by the use of a Lysis Buffer containing: 7.5 M Guanidine HCl, 25 mM TES, 10 mM EDTA, 0.05% Taurodeoxycholate, 1 mM 2-mercaptoethanol, pH 7.5 (all reagents Molecular Biology grade from Sigma, St. Louis, MO.). This buffer eliminated the necessity for grinding or icing samples and resulted in a stable preparation of DNA and RNA.

Cells were lysed in 1 mL lysis buffer per 10^8 cells/mL cell culture media (IMDM, Irvine Scientific; Irvine, CA) supplemented with 15% FBS (Hyclone; Logan, Utah). The lysate was extracted with equal volumes of phenol and chloroform/isoamyl alcohol. The aqueous phase was aspirated and re-extracted with an equal volume of chloroform /isoamyl alcohol. The aqueous phase was precipitated with 7/13 volumes 10M LiCl (all reagents Molecular Biology Grade from Sigma Chemicals, St. Louis, MO). The mRNA was prepared from the total RNA produced in the previous steps using a PolyATtract kit (Promega, Madison, WI).

3. RT-PCR Amplification

RT-PCR amplification of antigen sequences was performed on a Perkin-Elmer 2400 Thermal Cycler using a GeneAmp PCR kit (Perkin-Elmer/Roche Molecular Systems, Branchburg, NJ). Amplification was performed with 3 µL purified mRNA, 35 cycles, for the first amplification step of each reaction. The RT primer was designated 753RT, sequence TCGTTCATTCTCCTTAT (SEQ ID NO:9). The PCR primer for the first reaction was designated 42M, sequence GCTGGTAAATGTCCTCT (SEQ ID NO:10). For the nested PCR, 20 µL of product from the first PCR reaction was re-

amplified for 35 cycles using the 753RT primer and primer 412M, sequence ATGTAATGAGGGGTATC (SEQ ID NO:11). All primer concentrations were set at 0.2 μM, and the annealing temperature was set at 48°C.

4. Results

Gel electrophoresis of the first-step RT-PCR products is shown in Figure 1. Analysis of the second-step products by electrophoresis in TAE buffer (40 mM Tris-acetate, 20 mM EDTA, pH 8.3) on a 1 percent agarose gel revealed a band of the expected size, 341 bp, upon staining with ethidium bromide (Figure 2, lane 3). Amplification of the kit positive control PAW 109 gave the expected 311 base pair product. Re-amplification of this product with the kit DM152 and 151 primers was negative, a common result when re-amplifying PCR product without changing primers.

EXAMPLE V

CERVICAL CANCER

A. Cervical Specimen Handling and Disposal

1. Preparation of tubes for cervical sample collection and transportation:

Tubes: Corning polypropylene, 4mls, cat # SP:T4188-5, containing 100 μl of saline solution each, and were shipped to the clinical sites.

Cervical samples were collected using swabs. The swabs were inserted into the tubes and the tips broken off prior to covering the tubes for shipment.

2. Specimen Preparation prior to Assay:

Add 2 mls of 100 mM NaCl, 10 mM Tris-HCl (pH 8.0) containing 1 mM EDTA to 2 tubes and this buffer containing 0.1% Tween to the third tube. (The third sample is for investigating sample elution

from the swab and may be used for other extraction buffers as needed.)

Vortex tubes for 3-5 minutes.

Pull out the swab with forceps while squeezing to the side of the tube to let all fluids out of the swab.

Recap the tubes and centrifuge for 5 minutes at 3000 rpm in a table top centrifuge.

Serially dilute the supernatant to 1/8 and 1/16 with buffer.

Add 20 µl of protease inhibitor cocktail to each tube and 20 µl of the 10% sodium azide solution. Mix well and freeze remaining supernatants at -80°C prior to testing in ELISA.

B. ELISA

Sixty-three cervical samples were tested in several EIA combinations (Example IV.B.) including the X-52.1MAb/X-13.1 ALP sandwich EIA. The following are the assay procedure and results:

1. Assay Procedure

a. EIA plates (COSTAR-Hi binding) were coated with X-52.1 MAb at 5 µg/ml in Carb/Bicarb buffer (pH 9.6), 100 µl per well, and incubated overnight at 4°C.

b. Coating buffer was discarded and the plates were blocked with 250 µl per well with 2% BSA/PBS (pH 7.4) for 2 hrs at room temp.

c. Plates were then washed once with TBS containing 0.1% Tween-20.

d. Cervical samples were thawed at room temperature, centrifuged at 3000 rpm for 5 mins, then the supernatant was diluted to 1:20 in 25mM Tris-HCl, pH 7.8, in tubes and 100 µl of sample/diluent transferred per well to duplicate wells. The plates were sealed with plate covers and incubated at 37°C for 2 hrs.

e. Plates were washed 4x with TBS containing 0.1% Tween-20.

f. X13.1-AP conjugate was diluted to 2 µg/ml in 1% BSA/TBS (pH 7.4) and 100 µl transferred to each well. Plates were then covered and incubated at 37°C for 2 hrs.

g. Plates were washed 4x with 0.1%T/TBS.

h. A 1mg/ml solution of p-NPP/ 1M diethanolamine was made (pH 9.8), and 100 µl was transferred to each well. Plates were sealed with plate covers and incubated at 37°C for 1/2 hour.

i. Color development was stopped by adding 50 µl per well of 0.1M EDTA (pH 9.8).

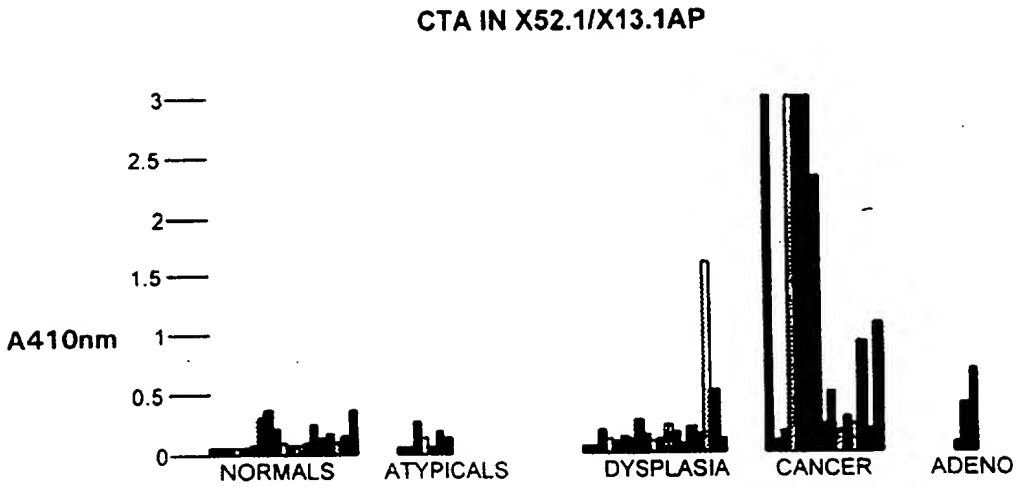
j. Plates were read at 410 nm on a Dynatech MR7000

2. Results

Results from the study of the cervical specimens with this assay are tabulated in Table 6 and presented graphically below.

Table 6

	X13.1MAb/ X52.1- AP
Sensitivity (cancers) n=15	73%
Specificity (normals) n=19	100%
% Adenocarcinoma above cutoff n=3	33%
% Dysplasia above cutoff n=19	16%
% Atypicals above cutoff n=7	0%
Cutoff (Mean OD of Normals +2SD)	0.201



EXAMPLE VI

PRODUCTION OF CFHRP IN CANCER

A. Production of CFHrp in Cancer Cell Lines

1. Detection of Antigen in Cell Culture Media by Immunossay

Cell culture media were tested for the presence of antigen (complement Factor H-related protein, CFHrp) using the sandwich enzyme immunoassay as described in Example IV.B. The media tested were those taken from cell cultures used for the preparation of total cellular RNA. After removal of the cultured cells, the remaining media free of cells were then diluted, as necessary, and tested in the EIA, as described. Control experiments involved the testing of fresh media, in particular those specified by ATCC or Clonetics Corporation (San Diego, CA) for the cell lines or primary cultures of interest. These were typically Modified Eagle's Media containing 10% fetal bovine serum (Sigma Chemical).

2. Detection of Message for Antigen in Cancer Cells by RT-PCR

cDNA was synthesized from mRNA present in preparations of total cellular RNA from cancer cell lines, using Reverse Transcriptase plus Random

Hexamer primers. The concentrations of components within the reaction mixture were as follows:

Component	Final Concentration
$MgCl_2$	5 mM
KCl	50 mM
Tris-HCl, pH 9.0	10 mM
Triton X-100	0.1% v/v
dGTP	1 mM
dATP	1 mM
dCTP	1 mM
dTTP	1 mM
RNAsin	1 U/μL
Random Hexamers	5 μM
MuLV Reverse Transcriptase	2.5 U/μL

Sample and DEPC-treated deionized water were added to bring the reaction volume to 20 μL. Between 1 and 5 μg of total RNA was added to each reaction mixture. Typically, 2 μg is sufficient. The cDNA reaction was allowed to proceed for 90 minutes at 42°C.

PCR of huCFH and huCFHrp mRNA was performed primarily with primer pair 42M and 1040RT (TCTGGATAATCACAAGGTTTC) (SEQ ID NO: 17), and primer pair 2910M (GTCAGACAGTTATCAGTATGGAGAAGAAG) (SEQ ID NO:18) and 3610RT (CTGTTTGGCTGTCCACCTTAATGCTATG) (SEQ ID NO:19). In addition, the presence of the correct internal sequences were confirmed with the primer pair 410M (ACATGTAATGAGGGGTATCAA) (SEQ ID NO:20) and 1040RT. The PCR master mix consisted of the following:

Component		Final Concentration
$MgCl_2$		3 mM
KCl		50 mM
Tris-HCl, pH 9.0		10 mM
Triton X-100		0.1% v/v
Taq DNA Polymerase		2.5 U/100 µL
Primer Pairs:		
	42M/1040RT	1-5 µM
or	2910M/3610RT	1-5 µM
or	410M/1040RT	1-5 µM

PCR was performed by adding 80 µL of master mix to each cDNA reaction tube. Thermal cycling was performed in a Perkin-Elmer (Foster City, CA) model 2400 cycler for 40 cycles. Positive results were determined by electrophoresis (e.g., at 90 volts for 90 min.) on 2% agarose gels, followed by staining with ethidium bromide, and destaining in deionized water.

For purposes of this application, moderate stringency hybridization and PCR amplification conditions are defined as those performed at the calculated melting temperature (Tm) of the probe/primer with the target. The recommended formula for calculating Tm, and its limitations, are well known in the art (i.e., are found in Sambrook, J., Fritsch, E.F. and T. Maniatis, *Molecular Cloning*, 2d Edition, Cold Spring Harbor Laboratory press, pp. 9.51-9.52, 1989). High stringency conditions are defined within this application as hybridization/amplification performed at least 4°C above the calculated Tm.

Using primer pair 42M/1040RT, CFHrp are detected at moderate (52°C) to high stringency (56°C) conditions, based on the homology of the cDNA to that of the primers identified in this application. Using primer pair 2910M/3610RT, CHFrp are detected at moderate (67°C) to high stringency (72°C) conditions, based on the homology of the cDNA to the primer pair. For internal cDNA sequence, using the primer pair 410M/1040RT, CFHrp are detected at moderate (48°C) to high stringency (56°C) conditions.

For PCR studies, DEPC-treated deionized water, Taq polymerase, RNAsin, and MuLV Reverse Transcriptase were from Promega Corporation (Madison, WI). Primers were synthesized by Midland Certified Reagents (Midland, TX) and were purified by anion-exchange chromatography. Agarose gels and ethidium bromide were from Sigma. All other reagents were obtained from Perkin-Elmer.

3. Results

a. Antigen Production and mRNA in Various Cell Lines

Table 7 illustrates that a wide variety of human cancer cells from established lines express CFHrp tumor antigen as determined by presence of immunologically active antigen in the cell culture media and the appropriate mRNA within the cells. Myeloid lines, a human colon cancer line, and normal human epithelial keratinocytes (a primary culture, not an established cell line) are negative for antigen expression by both assay methods used. In contrast, many bladder, renal, cervical and prostate cancer cell lines produce this tumor antigen.

Table 7

Production of huCFH by Various Cell Lines

Cell Line	Tumor Source	sEIA (Media)	RT-PCR
RCC7860	Renal Cell Ca.	Neg	++
ACHN	"	Neg	(+)
Pastor	"	+	++
769P	"	+	++
CAKI-1	Renal Clear Cell	Neg	(+)
HL60	Myeloid	Neg	Neg
LSI74T	Colon AdenoCa.	Neg	Neg
T24	TCC, Bladder	3+	++
5637	Primary Bladder CA	Neg	+
RT4	Papillary Bladder CA	3+	++

Cell Line	Tumor Source	sEIA (Media)	RT-PCR
J82	TCC, Bladder	3+	(+)
486P	TCC, Bladder	3+	++
HTB5	TCC, Bladder	Neg	+
HTB9	TCC, Bladder	2+	++
DU145	Prostate Ca.	ND	+
PC3	Prostate Ca.	ND	+
LNCaP	Prostate Ca.	ND	(+)
HeLaS3	Cervical AdenoCa.	4+	++
HTB33	Cervical	ND	+
C4I	Cervical	ND	+
DMEM/10%FBS	Cell Culture Media (not exposed to human cells)	Neg	NA
NHEK	Normal human epithelial keratinocytes, NOT an established cell line	Neg	Neg
X44.1	mouse hybridoma	Neg	Neg
X52.1	"	Neg	Neg
X13.2	"	Neg	Neg

NA, not applicable; ND, not determined

b. Analysis of RT-PCR Amplification Products

Total RNA was set at 3 μg and 40 cycles of amplification were performed. Annealing temperatures were set at 50°C for 42M1040RT pairs, 56°C for 410M1040RT and 70°C for 2910M3610RT. The 42M1040RT product expressed with mRNA from cervical adenocarcinoma HeLaS3 cells is of the expected size for a human CFH-derived product and includes the 5' UTR or CFH, to which the 42M primer hybridizes. A second upstream primer, designated 410M (ACATGTAATGAGGGGTATCAA) (SEQ ID NO:20) and also derived from the huCFH sequence (GenBank Accession number Y00716), also yields a product of expected size and restriction map from HeLaS3 cells. In addition, RT-PCR of total RNA from TCC bladder cancer cell line HTB9 yields a cDNA of an appropriate size

and restriction map using the 410M1040RT primer pair. The same primer pair yields no amplicon at the expected size for the colon adenocarcinoma line LS174T, myeloblastoma line HL-60 or normal human epithelial keratinocytes (NHEK). The experiments with preparations of HeLaS3 and HTB9 total RNA utilizing the 42M1040RT primer pair produced not only cDNA of the expected size, but also amplicons of unexpected sizes at 800 base pairs and, just above the limit of detectability, at 450, 480 and 1400 base pairs. In contrast, under the same conditions, the LS174T cell line produces only cDNA of incorrect sizes at 800, 450 and 420 base pairs, as well as an amplicon of 1200 base pairs which is at the limit of detectability.

Alterations in the CFH gene product are not restricted to the 5' end of the molecule. Although amplification of the downstream portion of the HeLaS3 cDNA with CFH primers 2910M (GTCAGACAGTTATCAGTATGGAGAAGAAG) (SEQ ID NO:18) and 3610RT (CTGTTTGGCTGTCCACCTTAATGCTATG) (SEQ ID NO:19) yielded amplicons of the expected size, the same primer pair yielded no amplicon with RNA from LS174T. Furthermore, both NHEK and HL-60 lines remained negative for the expression of CFHrp.

Figure 5 shows the gel electrophoresis of amplification products resulting from RT-PCR performed with three primer sets derived from human complement Factor H (lanes 1 to 10 beginning at the left side of the gel with the left side set of numbers 1-4 on the Figure representing lanes 1-4, the middle set of numbers 1-4 representing lanes 6-9 with lane 5 preceding, and the right side set of numbers 1-4 representing lanes 11-14 with lane 10 preceding). Lane 1:HTB-9 product with primers 1040RT and 42M; Lane 2: HeLaS3 product with primers 1040RT and 42M; Lane 3: NHEK product with primers 1040RT and 42M; Lane 4: LS174T product with primers 1040RT and 42M; Lane 6: HTB-9 product with primers 1040RT and 410M; Lane 7: HeLaS3 product with primers 1040RT and 410M; Lane 8: NHEK product with primers 1040RT and 410M; Lane 9: LS174T product with primers 1040RT and 410M; Lane 11: HTB-9 product with primers 3610RT and 2910M; Lane 12: HeLaS3 product with primers 3610RT and 2910M; Lane 13: NHEK product with primers 3610RT and

2910M; Lane 14: LS174T product with primers 3610RT and 2910M; Lanes 5 and 10: DNA molecular weight markers.

B. Method of Cloning cDNA Coding for CFHrp

A cDNA of the appropriate size and restriction pattern for human CFH was prepared from mRNA isolated from the HeLaS3 human cervical adenocarcinoma cell line. This cDNA, prepared with primers 42M and 1040RT, was blunt-end cloned into pBluescript SK (Stratagene, La Jolla, CA).

1. Cloning and Sequencing

a. PCR product, in a total volume of 73 µL, was purified using a Prep-A-Gene DNA purification kit (Stratagene). To the product in solution, 360 µL of the purification kit binding buffer and 20 µL of Prep-A-Gene DNA binding matrix were added. Purification was then performed according to the protocol described in the package insert.

DNA concentration was estimated by running 2 µL of the purified material on a 2% agarose gel (Sigma) and comparing its intensity to the intensity of a standard (Sigma) of known concentration.

b. To ensure that the PCR product had blunt ends, the ends were filled in using the PCR Polishing Kit (Stratagene). 1 µL of dNTP mix and 1 µL of Pfu Polymerase were added to 7 µL of purified PCR product to which had been added 1 µL of 10X Pfu Polymerase Buffer. The reaction mixture was incubated at 72°C for 30 minutes. Polished reactions were stored at -80°C until use. No further purification was required.

c. In order to yield blunt ends, 20 µg of pBluescnpt SK- was digested with 30 units of Eco RV in a 100 µL reaction volume containing 6 mM Tris-HCl, pH 7.9, 6 mM $MgCl_2$, 150 mM NaCl, and 1 mM DTT. The reaction mixture was incubated overnight (16-18 hours) at 37°C.

d. Phenol extraction of the digested plasmid was performed by adding an equal volume of Buffer-Saturated Phenol (Sigma) and vortexing to mix.

The mixture was centrifuged at 5000 rpm for 5 minutes to separate the phases. The upper aqueous phase was removed and transferred to a new tube.

To the aqueous solution were added 50 µL phenol and 50 µL chloroform:isoamyl alcohol (24:1). The phases were mixed by vortexing and separated as above. The upper aqueous phase was removed and transferred to a new tube. This aqueous phase was again extracted with 100 ul of chloroform:isoamyl alcohol. The phases were separated as before, and the upper aqueous phase was removed and transferred to a fresh tube.

e. In order to precipitate DNA from the aqueous solution, 0.5 volumes of 7.5 M ammonium acetate (pH 5.5) and 2 volumes of ethanol were added and incubated at -20°C for 1 hour. DNA was pelleted by centrifugation at 12,000xG for 20 minutes. The ethanol was carefully decanted. The DNA pellet was washed with 500 µL of 70% ethanol and then pelleted and supernatant decanted as above.

The DNA pellet was allowed to air dry by inverting the tube until just dry by visual inspection and was then resuspended in water (Molecular Biology Grade, Sigma).

f. 10 µg of digested pBluescript DNA was dephosphorylated with 500 units of Calf Intestinal Alkaline Phosphatase by incubating at 37°C for 15 minutes in a total volume of 100 µL, containing 0.05 M Tris-HCl, pH 9.3, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$ and 1 mM Spermidine. Another 500 units of alkaline phosphatase was added and reaction was incubated at 56°C for 45 minutes. The reaction mixture was then incubated at 65°C for 1 hour to inactivate the alkaline phosphatase. The reaction mixture was extracted with phenol and chloroform and then precipitated with ethanol, as described in step (d).

g. Ligation of vector and insert was performed using the two-step ligation procedure described by S. Damak and D.W. Bullock (*BioTechniques 15*(3):448-452, 1993). In the first step of the ligation process, vector and insert were mixed together at vector:insert ratio of 1:4. 150 nmol of polished insert was mixed with 36 nmol of vector in a 10 µL volume containing 0.05 M Tris, 0.025 M $MgCl_2$, 0.5 mM ATP, 1 mM DTT, 1 unit of T4 DNA Ligase and 10 units of EcoRV. The reaction was incubated at room temperature for 2 hours For the second part of the ligation process, 190 µL of a solution containing 0.05 M Tris, 0.025 M $MgCl_2$, 0.5 mM ATP, 1 mM DTT, and 19 units of T4 DNA Ligase was added to the reaction mixture from the first step and incubated at room temperature overnight (16-18 hours).

h. Transformation of competent *E. coli* was initiated by first thawing frozen competent DH5 (Stratagene) cells in an ice water bath.

1.7 mL microfuge tubes were labelled and placed on ice. When thawed, 50 µL of the competent cells was pipetted into the labelled tubes. 5 µL of the ligation reaction mixture was added to the tube containing the competent cells. The tube was flicked briefly to mix and incubated on ice for 30 minutes. This tube containing the competent cell/ligation mixture was then incubated at 37°C for 20 seconds. A 0.95 mL volume of LB broth (Sigma) was added and tubes were incubated at 37°C for 1 hour with shaking at 225 rpm. 200 µL of cell mixture was spread, using sterile spreaders, onto the surface of an LB agar plate containing 50 µg/ml Ampicillin, 25 µg/ml X-Gal and 60 µg/ml IPTG (all reagents from Sigma). Plates were covered and incubated at 37°C overnight (16-18 hours). White colonies were selected and screened by purifying the plasmid DNA and then performing restriction analysis.

i. All DNA sequencing was performed on ABI Prism sequencers (Perkin-Elmer, Foster City, CA) in the laboratory of Dr. Leroy Hood, Center for Molecular Biotechnology, University of Washington, Seattle, WA.

Results

Table 8 is a tabulation of observed differences between the clone sequences (for DNA encoding complement Factor H-related protein) and the published sequence for DNA encoding human complement Factor H (CFH).

Table 8

Apparent Differences Between Clone and Human CFH Sequences

Clone	RNA Source	DNA Position Number	DNA Change	Mature Protein Position Number	Amino Acid Change
pRBB9FH410#2.1	HTB 9	672	A to G	181	S to G
pRBS3FH2910#2.1	Hela S3	3096	G to T	989	V to L
pRBS3FH2910#3.1	Hela S3	3046	T to C	972	L to P
		3094	A to G	988	D to V
		3096	G to T	989	V to L
		3142	A to G	1004	Y to C
		3144	A to C	1005	K to Q
pRBS3FH2910#4.1	Hela S3	3053	A to G	974	No Change
		3096	G to T	989	V to L
		3115	A to C	995	Q to P
pZS3FH2576#3	Hela S3	2621	A to G	830	No Change
		2746	G to T	872	S to I
pZS3FH2576#1/11	Hela S3	2746	G to T	872	S to I

Figure 6A shows a partial DNA sequence from clone pRBB9FH410 and Figure 6B the corresponding amino acid sequence, as compared to the DNA and amino acid sequences for human CFH. Figure 7A shows three partial DNA sequences from

clone pRBS3FH2910 and Figure 7B the corresponding amino acid sequences, as compared to the DNA and amino acid sequences for human CFH. Figure 8A shows two partial DNA sequences from clone pZS3FH2576 and Figure 8B the corresponding amino acid sequences, as compared to the DNA and amino acid sequences for human CFH.

2. Generation and Use of Riboprobes

a. A riboprobe for use in *in situ* hybridization was prepared from the same clone. Restriction digestion of the clone was performed with Ava II (New England Biolabs, Beverly, MA) following the manufacturer's instructions. The resulting cDNA corresponded to map positions 457-1057 of huCFH (GenBank sequence number YM00716). The cDNA product was purified by electrophoresis on a 1% agarose TAE gel (Sigma). A digoxin-labelled ribonucleic acid antisense probe was prepared with T7 RNA polymerase, using a commercial kit (Boehringer-Mannheim, Indianapolis, IN) and following the instructions in the package insert. Unlabelled RNA for probe competition was synthesized in a similar manner using a kit from Ambion (Austin, TX) following the manufacturer's instructions.

b. The resulting Riboprobes were purified by precipitation with an equal volume of absolute ethanol and were then redissolved in ribonuclease-free water to final concentrations of either 101 nM for the digoxigenin-labelled probe or 10 uM for the unlabelled probe.

c. Pathology tissue specimens were prepared for staining by snap freezing in liquid nitrogen. The frozen pellets were sectioned on a cryostat microtome (Bartles & Stout) and then fixed in (-20°C) acetone (Sigma) for 15 minutes. Fixed sections were placed on a slide and kept wet in APK buffer (Ventana, Tucson, AZ) until the hybridization process was begun.

d. For tissue staining, 1 µL of stock riboprobe was diluted into 500 µL of hybridization solution, consisting of 2X Denhardt's solution supplemented with 60% (w/v) formamide, 12.5% dextran sulfate, 10 mM Tris, 1 mM EDTA, 1 mM DTT, 375 mM NaCl, 0.3% Triton X100, and containing 2 mg tRNA (all reagents from Sigma). Final concentration of the digoxigenin-labelled RNA probe was 0.3 nM.

e. Staining was performed on an ES GenII slide processor (Ventana, Tucson, AZ), using reagent packs and buffers from the manufacturer, with detection by HRP-conjugated anti-mouse antibody. Hybridization solution containing the riboprobe was applied manually and processed wet. Following denaturation for 2 minutes at 65°C, hybridization was carried out at 40°C for 120 minutes. Three washes were performed at 55°C sequentially with 1X SSC (150 mM sodium chloride, 15 mM sodium citrate, pH 7.0-7.5) 0.5X SSC and 0.1X SSC. Slides were then reacted with anti-digoxigenin antibody following the manufacturer's instructions (Boehringer Mannheim).

f. A specificity control was performed by application of riboprobe stock containing a 100-fold excess of riboprobe that had not been labeled with digoxigenin.

Results

The target tissues subjected to staining with the riboprobe were serial sections from normal and cancerous human bladder (transitional cells) and from normal and cancerous human prostate. All tissue sections, both normal and cancerous, were from a single bladder or a single prostate.

Specificity of tissues staining was established by competition of digoxigenin labelled probe binding with a 100-fold excess of unlabelled probe. Only sections from TCC+ bladder cancer stained with the HeLaS3 generated probe sequence.

EXAMPLE VII

INHIBITION OF ANTIGEN BIOLOGICAL ACTIVITY BY MABS

A. *In Vitro* Protection of C3b by Anti-CFH Related Protein MAbs

As shown in Example III.F the complement Factor H-related activity of antigen can be mimicked by complement Factor H itself. For experimental clarity, therefore, Factor H and Factor I were used to degrade C3b and illustrate the protective actions of anti-Factor H-related protein MAbs.

Reactions were performed by incubating 1 μg of Factor H with either 15 or 30 μg of each of three MOF MAbs (X52.1; X87.2; X13.2), in 20 μL of phosphate-buffered saline for 30 minutes at 37°C, followed by the addition of 7.5 μg of C3b and 5 μg of Factor I into each reaction tube (final reaction volume 32.5 μL). (C3b was generated from C3 by the method of Pangburn, M.K., and Mueller-Eberhard, H.J., *Biochemistry 22*:178-185, 1983.) The mixture was then incubated on a rotator at 37°C for 1 hour. Results were determined by SDS-PAGE of the reaction mixtures under reducing conditions (50 mM DTT) on a 4-12% gradient gel (Novex, San Diego, CA). (Unless specified otherwise, all reagents are from Sigma, St. Louis; MO.) The gel was scanned with a GelDoc scanner (BioRad, Hercules, CA). The intensities of the bands measured in this way were converted to percentage of C3b remaining. The control lane containing the reaction mixture in the absence of MAb was used to represent 100 percent degradation, while the lane containing the reaction mixture with no Factor H was used to represent 0 percent degradation.

Results

The results derived from scanning the gel are summarized in Table 8. These results demonstrate that MAbs X13.2 and X87.2 are able to block the Factor H-related protein antigen-mediated degradation of complement component C3b *in vitro*.

B. Activation of Cell Lysis by the Alternate Complement Pathway by MAbs Specific for the Antigen

Standard guinea pig complement was treated with 5 mM EGTA to chelate calcium. Then 5 mM $MgCl_2$, which is required for the activity of the alternative complement pathway (ACP), was added. The mixture was incubated for 20 minutes at 37°C, then added to 7 x 10^9 rabbit red blood cells (RBC) and further incubated at 37°C. After 45 minutes and again after 117 minutes, hemolysis was determined by measuring the A_{450} and comparing the values to those determined for control reactions, which had received either no complement or no MAb. Measurements were performed on a Dynatech (Chantilly, VA) MR5000 96-well microplate reader. Hemolysis was determined in the absence or presence of MAb X52.1. When included, the MAb was used at a concentration in the reaction mixture of 10 nM or 30 nM (Figure 3). All reagents and materials were purchased from Sigma.

A second experiment was performed under the same conditions as the RBC lysis, but the target cells were HL-60 (1 x 10^8 cells), a human myeloid cell line. MAb concentration was set to 10 nM, and lysis was read after 120 minutes as described above (Figure 4).

Results

Although MAb X52.1 was not effective in the format used in Example VII.A above, perhaps due to a lower affinity for Factor H, it was highly effective in promoting lysis of RBCs or HL-60 cells. RBC lysis was dependent on the concentration of MAb and the duration of incubation. Since X52.1 does not bind to RBCs or HL-60 cells, and since HL-60 cells do not produce Factor H, the mechanism of action of cell lysis must be the binding of X52.1 to the Factor H in the added guinea pig complement and resulting inhibition of the C3 stabilization activity of the Factor H.

In summary, MAb X52.1, which specifically binds antigen and CFH, but does not bind red blood cells, Factor I or C3b, can promote the ACP-mediated lysis of RBCs. The concentrations required to do this are achievable physiologically (10 nM, or approximately 1.5 μg/mL).

Table 9

Inhibition of C3b Degradation in the Presence of Anti-Antigen MAbs

Sample	Quantity of Sample	C3b Remaining (Percent)
Control, No MAb	Standard	0 -
X52.1	15 µg	0
X52.1	30 µg	0
X87.2	15 µg	24.7
X87.2	30 µg	54.9
X13.2	15 µg	62.0
X13.2	30 µg	54.0
Control, No Factor H	Standard	100

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually incorporated by reference.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Kinders, Robert J.
Enfield, David L.
Hass, G. Michael

(ii) TITLE OF INVENTION: METHODS AND COMPOSITIONS FOR SCREENING FOR OR MODULATING A TUMOR ASSOCIATED ANTIGEN

(iii) NUMBER OF SEQUENCES: 38

(iv) CORRESPONDENCE ADDRESS:
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE: 09-APR-1997
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(viii) ATTORNEY/AGENT INFORMATION:
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(C) REFERENCE/DOCKET NUMBER: 130001.404Pc

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Glu Asp Cys Asn Xaa Leu Pro Pro Arg Xaa Asn Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gly Pro Tyr Phe Pro Val Ala Val Gly Lys Tyr Tyr Xaa Tyr Tyr Xaa
1 5 10 15

Asp

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Arg Pro Tyr Phe Pro Val Ala Val Gly Lys Tyr Tyr Ser Xaa Tyr Xaa
1 5 10 15

Asp Glu Xaa Phe Xaa Xaa Xaa Ser
20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ser Ser Gln Glu Ser Tyr Ala His Gly Thr Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Glu Asp Cys Asn Glu Leu Pro Pro Xaa Arg Asn Thr Glu Ile Leu Xaa
1 5 10 15

Gly Ser Trp Asp
20

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Arg Pro Tyr Phe Pro Val Val Ala Val Gly Lys Tyr Tyr Ser Tyr Tyr
1 5 10 15

Xaa Asp Glu His Phe Glu Xaa Pro
20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ser Leu Gly Asn Val Ile Met Val Gly Arg Lys Gly Glu Trp Val Ala
1 5 10 15

Leu Asn Pro Leu Arg Lys
20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Arg Pro Tyr Phe Pro Val Ala Val Gly Lys Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TCGTTCATTC TCCTTAT 17

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCTGGTAAAT GTCCTCT 17

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATGTAATGAG GGTATC 17

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Arg Pro Tyr Phe Pro Val Ala Val Gly Lys Tyr Tyr Ser Tyr Tyr Cys
1 5 10 15

Asp

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Arg Pro Tyr Phe Pro Val Ala Val Gly Lys Tyr Tyr Ser Tyr Tyr Cys
1 5 10 15

Asp Glu His Phe Glu Thr Pro Ser
20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Glu Asp Cys Asn Glu Leu Pro Pro Arg Arg Asn Thr Glu Ile Leu Thr
1 5 10 15

Gly Ser Trp Ser Asp
20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Arg Pro Tyr Phe Pro Val Ala Val Gly Lys Tyr Tyr Ser Tyr Tyr Cys
1 5 10 15

Asp Glu His Phe Glu Thr Pro
20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Ser Leu Gly Asn Val Ile Met Val Cys Arg Lys Gly Glu Trp Val Ala
1 5 10 15

Leu Asn Pro Leu Arg Lys
20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TCTGGATAAT CACAAGGTTT C 21

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GTCAGACAGT TATCAGTATG GAGAAGAAG 29

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CTGTTTGGCT GTCCACCTTA ATGCTATG 28

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ACATGTAATG AGGGGTATCA A 21

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 649 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ACATGTAATG AGGGGTATCA ATTGCTAGGT GAGATTAATT ACCGTGAATG TGACACAGAT 60

GGATGGACCA ATGATATTCC TATATGTGAA GTTGTGAAGT GTTTACCAGT GACAGCACCA 120

GAGAATGGAA AAATTGTCAG TAGTGCAATG GAACCAGATC GGGAATACCA TTTTGGACAA 180

GCAGTACGGT TTGTATGTAA CTCAGGCTAC AAGATTGAAG GAGATGAAGA AATGCATTGT 240

TCAGACGATG GTTTTTGGAG TAAAGAGAAA CCAAAGTGTG TGGAAATTTC ATGCAAATCC 300

CCAGATGTTA TAAATGGATC TCCTATATCT CAGAAGATTA TTTATAAGGA GAATGAACGA 360

TTTCAATATA AATGTAACAT GGGTTATGAA TACAGTGAAA GAGGAGATGC TGTATGCACT 420

GAATCTGGAT GGCGTCCGTT GCCTTCATGT GAAGAAAAAT CATGTGATAA TCCTTATATT 480

CCAAATGGTG ACTACTCACC TTTAAGGATT AAACACAGAA CTGGAGATGA AATCACGTAC 540

CAGTGTAGAA ATGGTTTTTA TCCTGCAACC CGGGGAAATA CAGCCAAATG CACAAGTACT 600

GGCTGGATAC CTGCTCCGAG ATGTACCTTG AAACCTTGTG ATTATCCAG 649

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 581 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

ACATGTAATG AGGGGTATCA ATTGCTAGGT GAGATTAATT ACCGTGAATG TGACACAGAT 60

GGATGGACCA ATGATATTCC TATATGTGAA GTTGTGAAGT GTTTACCAGT GACAGCACCA 120

GAGAATGGAA AAATTGTCAG TAGTGCAATG GAACCAGATC GGGAATACCA TTTTGGACAA 180

GCAGTACGGT TTGTATGTAA CTCAGGCTAC AAGATTGAAG GAGATGAAGA AATGCATTGT 240

TCAGACGATG GTTTTTGGGG TAAAGAGAAA CCAAAGTGTG TGGAAATTTC ATGCAAATCC 300

CCAGATGTTA TAAATGGATC TCCTATATCT CAGAAGATTA TTTATAAGGA GAATGAACGA 360

TTTCAATATA AATGTAACAT GGGTTATGAA TACAGTGAAA GAGGAGATGC TGTATGCACT 420

GAATCTGGAT GGCGTCCGTT GCCTTCATGT GAAGAAAAAT CATGTGATAA TCCTTATATT 480

CCAAATGGTG ACTACTCACC TTTAAGGATT AAACACAGAA CTGGAGATGA AATCACGTAC 540

CAGTGTAGAA ATGGTTTTTA TCCTGCAACC CGGGGAAATA C 581

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 240 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Phe Thr Leu Thr Gly Gly Asn Val Phe Glu Tyr Gly Val Lys Ala Val
1 5 10 15

Tyr Thr Cys Asn Glu Gly Tyr Gln Leu Leu Gly Glu Ile Asn Tyr Arg
20 25 30

Glu Cys Asp Thr Asp Gly Trp Thr Asn Asp Ile Pro Ile Cys Glu Val
35 40 45

Val Lys Cys Leu Pro Val Thr Ala Pro Glu Asn Gly Lys Ile Val Ser
50 55 60

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Ser Ala Met Glu Pro Asp Arg Glu Tyr His Phe Gly Gln Ala Val Arg
65                  70                  75                  80

Phe Val Cys Asn Ser Gly Tyr Lys Ile Glu Gly Asp Glu Glu Met His
                85                  90                  95

Cys Ser Asp Asp Gly Phe Trp Ser Lys Glu Lys Pro Lys Cys Val Glu
            100                 105                 110

Ile Ser Cys Lys Ser Pro Asp Val Ile Asn Gly Ser Pro Ile Ser Gln
        115                 120                 125

Lys Ile Ile Tyr Lys Glu Asn Glu Arg Phe Gln Tyr Lys Cys Asn Met
    130                 135                 140

Gly Tyr Glu Tyr Ser Glu Arg Gly Asp Ala Val Cys Thr Glu Ser Gly
145                 150                 155                 160

Trp Arg Pro Leu Pro Ser Cys Glu Glu Lys Ser Cys Asp Asn Pro Tyr
                165                 170                 175

Ile Pro Asn Gly Asp Tyr Ser Pro Leu Arg Ile Lys His Arg Thr Gly
            180                 185                 190

Asp Glu Ile Thr Tyr Gln Cys Arg Asn Gly Phe Tyr Pro Ala Thr Arg
        195                 200                 205

Gly Asn Thr Ala Lys Cys Thr Ser Thr Gly Trp Ile Pro Ala Pro Arg
    210                 215                 220

Cys Thr Leu Lys Pro Cys Asp Tyr Pro Asp Ile Lys His Gly Gly Leu
225                 230                 235                 240
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 216 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Thr Cys Asn Glu Gly Tyr Gln Leu Leu Gly Glu Ile Asn Tyr Arg Glu
1               5                   10                  15

Cys Asp Thr Asp Gly Trp Thr Asn Asp Ile Pro Ile Cys Glu Val Val
            20                  25                  30

Lys Cys Leu Pro Val Thr Ala Pro Glu Asn Gly Lys Ile Val Ser Ser
        35                  40                  45

Ala Met Glu Pro Asp Arg Glu Tyr His Phe Gly Gln Ala Val Arg Phe
    50                  55                  60

Val Cys Asn Ser Gly Tyr Lys Ile Glu Gly Asp Glu Glu Met His Cys
65                  70                  75                  80

Ser Asp Asp Gly Phe Trp Gly Lys Glu Lys Pro Lys Cys Val Glu Ile
                85                  90                  95
```

Ser Cys Lys Ser Pro Asp Val Ile Asn Gly Ser Pro Ile Ser Gln Lys
100 105 110

Ile Ile Tyr Lys Glu Asn Glu Arg Phe Gln Tyr Lys Cys Asn Met Gly
115 120 125

Tyr Glu Tyr Ser Glu Arg Gly Asp Ala Val Cys Thr Glu Ser Gly Trp
130 135 140

Arg Pro Leu Pro Ser Cys Glu Glu Lys Ser Cys Asp Asn Pro Tyr Ile
145 150 155 160

Pro Asn Gly Asp Tyr Ser Pro Leu Arg Ile Lys His Arg Thr Gly Asp
165 170 175

Glu Ile Thr Tyr Gln Cys Arg Asn Gly Phe Tyr Pro Ala Thr Arg Gly
180 185 190

Asn Thr Ala Lys Cys Thr Ser Thr Gly Trp Ile Pro Ala Pro Arg Cys
195 200 205

Thr Leu Lys Pro Cys Asp Tyr Pro
210 215

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 767 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CATGGTGTTG TAGCTCACAT GTCAGACAGT TATCAGTATG GAGAAGAAGT TACGTACAAA 60

TGTTTTGAAG GTTTTGGAAT TGATGGGCCT GCAATTGCAA AATGCTTAGG AGAAAAATGG 120

TCTCACCCTC CATCATGCAT AAAAACAGAT TGTCTCAGTT TACCTAGCTT TGAAAATGCC 180

ATACCCATGG GAGAGAAGAA GGATGTGTAT AAGGCGGGTG AGCAAGTGAC TTACACTTGT 240

GCAACATATT ACAAAATGGA TGGAGCCAGT AATGTAACAT GCATTAATAG CAGATGGACA 300

GGAAGGCCAA CATGCAGAGA CACCTCCTGT GTGAATCCGC CCACAGTACA AAATGCTTAT 360

ATAGTGTCGA GACAGATGAG TAAATATCCA TCTGGTGAGA GAGTACGTTA TCAATGTAGG 420

AGCCCTTATG AAATGTTTGG GGATGAAGAA GTGATGTGTT TAAATGGAAA CTGGACGGAA 480

CCACCTCAAT GCAAAGATTC TACAGGAAAA TGTGGGCCCC CTCCACCTAT TGACAATGGG 540

GACATTACTT CATTCCCGTT GTCAGTATAT GCTCCAGCTT CATCAGTTGA GTACCAATGC 600

CAGAACTTGT ATCAACTTGA GGGTAACAAG CGAATAACAT GTAGAAATGG ACAATGGTCA 660

GAACCACCAA AATGCTTACA TCCGTGTGTA ATATCCCGAG AAATTATGGA AAATTATAAC 720

ATAGCATTAA GGTGGACAGC CAAACAGAAG CTTTATTCGA GAACAGG 767

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 532 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GTCAGACAGT TATCAGTATG GAGAAGAAGT TACGTACAAA TGTTTTGAAG GTTTTGGAAT 60

TGATGGGCCT GCAATTGCAA AATGCTTAGG AGAAAAATGG TCTCACCCTC CATCATGCAT 120

AAAAACAGAT TGTCTCAGTT TACCTAGCTT TGAAAATGCC ATACCCATGG GAGAGAAGAA 180

GGATTTGTAT AAGGCGGGTG AGCAAGTGAC TTACACTTGT GCAACATATT ACAAAATGGA 240

TGGAGCCAGT AATGTAACAT GCATTAATAG CAGATGGACA GGAAGGCCAA CATGCAGAGA 300

CACCTCCTGT GTGAATCCGC CCACAGTACA AAATGCTTAT ATAGTGTCGA GACAGATGAG 360

TAAATATCCA TCTGGTGAGA GAGTACGTTA TCAATGTAGG AGCCCTTATG AAATGTTTGG 420

GGATGAAGAA GTGATGTGTT TAAATGGAAA CTGGACGGAA CCACCTCAAT GCAAAGATTC 480

TACAGGAAAA TGTGGGCCCC CTCCACCTAT TGACAATGGG GACATTACTT CA 532

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 688 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GTCAGACAGT TATCAGTATG GAGAAGAAGT TACGTACAAA TGTTTTGAAG GTTTTGGAAT 60

TGATGGGCCT GCAATTGCAA AATGCTTAGG AGAAAAATGG TCTCACCCTC CATCATGCAT 120

AAAAACAGAT TGTCCCAGTT TACCTAGCTT TGAAAATGCC ATACCCATGG GAGAGAAGAA 180

GGTTTTGTAT AAGGCGGGTG AGCAAGTGAC TTACACTTGT GCAACATATT GCCAAATGGA 240

TGGAGCCAGT AATGTAACAT GCATTAATAG CAGATGGACA GGAAGGCCAA CATGCAGAGA 300

CACCTCCTGT GTGAATCCGC CCACAGTACA AAATGCTTAT ATAGTGTCGA GACAGATGAG 360

TAAATATCCA TCTGGTGAGA GAGTACGTTA TCAATGTAGG AGCCCTTATG AAATGTTTGG 420

GGATGAAGAA GTGATGTGTT TAAATGGAAA CTGGACGGAA CCACCTCAAT GCAAAGATTC 480

TACAGGAAAA TGTGGGCCCC CTCCACCTAT TGACAATGGG GACATTACTT CATTCCCGTT 540

GTCAGTATAT GCTCCAGCTT CATCAGTTGA GTACCAATGC CAGAACTTGT ATCAACTTGA 600

GGGTAACAAG CGAATAACAT GTAGAAATGG ACAATGGTCA GAACCACCAA AATGCTTACA 660

TCCGTGTGTA ATATCCCGAG AAATTATG 688

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 590 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GTTTGCCTAG CTTTGAAAAT GCCATACCCA TGGGAGAGAA GAAGGATTTG TATAAGGCGG 60

GTGAGCCAGT GACTTACACT TGTGCAACAT ATTACAAAAT GGATGGAGCC AGTAATGTAA 120

CATGCATTAA TAGCAGATGG ACAGGAAGGC CAACATGCAG AGACACCTCC TGTGTGAATC 180

CGCCCACAGT ACAAAATGCT TATATAGTGT CGAGACAGAT GAGTAAATAT CCATCTGGTG 240

AGAGAGTACG TTATCAATGT AGGAGCCCTT ATGAAATGTT TGGGGATGAA GAAGTGATGT 300

GTTTAAATGG AAACTGGACG GAACCACCTC AATGCAAAGA TTCTACAGGA AAATGTGGGC 360

CCCCTCCACC TATTGACAAT GGGGACATTA CTTCATTCCC GTTGTCAGTA TATGCTCCAG 420

CTTCATCAGT TGAGTACCAA TGCCAGAACT TGTATCAACT TGAGGGTAAC AAGCGAATAA 480

CATGTAGAAA TGGACAATGG TCAGAACCAC CAAAATGCTT ACATCCGTGT GTAATATCCC 540

GAGAAATTAT GGAAAATTAT AACATAGCAT TAAGGTGGAC AGCCAAACAG 590

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 290 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Gly Phe Arg Ile Ser Glu Glu Asn Glu Thr Thr Cys Tyr Met Gly Lys
1 5 10 15

Trp Ser Ser Pro Pro Gln Cys Glu Gly Leu Pro Cys Lys Ser Pro Pro
20 25 30

Glu Ile Ser His Gly Val Val Ala His Met Ser Asp Ser Tyr Gln Tyr
35 40 45

Gly Glu Glu Val Thr Tyr Lys Cys Phe Glu Gly Phe Gly Ile Asp Gly
50 55 60

Pro Ala Ile Ala Lys Cys Leu Gly Glu Lys Trp Ser His Pro Pro Ser
65 70 75 80

Cys Ile Lys Thr Asp Cys Leu Ser Leu Pro Ser Phe Glu Asn Ala Ile
85 90 95

Pro Met Gly Glu Lys Lys Asp Val Tyr Lys Ala Gly Glu Gln Val Thr
100 105 110

Tyr Thr Cys Ala Thr Tyr Tyr Lys Met Asp Gly Ala Ser Asn Val Thr
115 120 125

Cys Ile Asn Ser Arg Trp Thr Gly Arg Pro Thr Cys Arg Asp Thr Ser
130 135 140

Cys Val Asn Pro Pro Thr Val Gln Asn Ala Tyr Ile Val Ser Arg Gln
145 150 155 160

Met Ser Lys Tyr Pro Ser Gly Glu Arg Val Arg Tyr Gln Cys Arg Ser
165 170 175

Pro Tyr Glu Met Phe Gly Asp Glu Glu Val Met Cys Leu Asn Gly Asn
180 185 190

Trp Thr Glu Pro Pro Gln Cys Lys Asp Ser Thr Gly Lys Cys Gly Pro
195 200 205

Pro Pro Pro Ile Asp Asn Gly Asp Ile Thr Ser Phe Pro Leu Ser Val
210 215 220

Tyr Ala Pro Ala Ser Ser Val Glu Tyr Gln Cys Gln Asn Leu Tyr Gln
225 230 235 240

Leu Glu Gly Asn Lys Arg Ile Thr Cys Arg Asn Gly Gln Trp Ser Glu
245 250 255

Pro Pro Lys Cys Leu His Pro Cys Val Ile Ser Arg Glu Ile Met Glu
260 265 270

Asn Tyr Asn Ile Ala Leu Arg Trp Thr Ala Lys Gln Lys Leu Tyr Ser
275 280 285

Arg Thr
290

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 177 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ser Asp Ser Tyr Gln Tyr Gly Glu Glu Val Thr Tyr Lys Cys Phe Glu
1 5 10 15

Gly Phe Gly Ile Asp Gly Pro Ala Ile Ala Lys Cys Leu Gly Glu Lys
20 25 30

Trp Ser His Pro Pro Ser Cys Ile Lys Thr Asp Cys Leu Ser Leu Pro
35 40 45

Ser Phe Glu Asn Ala Ile Pro Met Gly Glu Lys Lys Asp Leu Tyr Lys
50 55 60

Ala Gly Glu Gln Val Thr Tyr Thr Cys Ala Thr Tyr Tyr Lys Met Asp
65 70 75 80

Gly Ala Ser Asn Val Thr Cys Ile Asn Ser Arg Trp Thr Gly Arg Pro
85 90 95

Thr Cys Arg Asp Thr Ser Cys Val Asn Pro Pro Thr Val Gln Asn Ala
100 105 110

Tyr Ile Val Ser Arg Gln Met Ser Lys Tyr Pro Ser Gly Glu Arg Val
115 120 125

Arg Tyr Gln Cys Arg Ser Pro Tyr Glu Met Phe Gly Asp Glu Glu Val
130 135 140

Met Cys Leu Asn Gly Asn Trp Thr Glu Pro Pro Gln Cys Lys Asp Ser
145 150 155 160

Thr Gly Lys Cys Gly Pro Pro Pro Pro Ile Asp Asn Gly Asp Ile Thr
165 170 175

Ser

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 229 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Ser Asp Ser Tyr Gln Tyr Gly Glu Glu Val Thr Tyr Lys Cys Phe Glu
1 5 10 15

Gly Phe Gly Ile Asp Gly Pro Ala Ile Ala Lys Cys Leu Gly Glu Lys
20 25 30

Trp Ser His Pro Pro Ser Cys Ile Lys Thr Asp Cys Pro Ser Leu Pro
35 40 45

Ser Phe Glu Asn Ala Ile Pro Met Gly Glu Lys Lys Val Leu Tyr Lys
50 55 60

Ala Gly Glu Gln Val Thr Tyr Thr Cys Ala Thr Tyr Cys Gln Met Asp
65 70 75 80

Gly Ala Ser Asn Val Thr Cys Ile Asn Ser Arg Trp Thr Gly Arg Pro
85 90 95

Thr Cys Arg Asp Thr Ser Cys Val Asn Pro Pro Thr Val Gln Asn Ala
100 105 110

Tyr Ile Val Ser Arg Gln Met Ser Lys Tyr Pro Ser Gly Glu Arg Val
115 120 125

Arg Tyr Gln Cys Arg Ser Pro Tyr Glu Met Phe Gly Asp Glu Glu Val
130 135 140

Met Cys Leu Asn Gly Asn Trp Thr Glu Pro Pro Gln Cys Lys Asp Ser
145 150 155 160

Thr Gly Lys Cys Gly Pro Pro Pro Pro Ile Asp Asn Gly Asp Ile Thr
165 170 175

Ser Phe Pro Leu Ser Val Tyr Ala Pro Ala Ser Ser Val Glu Tyr Gln
180 185 190

Cys Gln Asn Leu Tyr Gln Leu Glu Gly Asn Lys Arg Ile Thr Cys Arg
195 200 205

Asn Gly Gln Trp Ser Glu Pro Pro Lys Cys Leu His Pro Cys Val Ile
210 215 220

Ser Arg Glu Ile Met
225

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 196 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Leu Pro Ser Phe Glu Asn Ala Ile Pro Met Gly Glu Lys Lys Asp Leu
1 5 10 15

Tyr Lys Ala Gly Glu Pro Val Thr Tyr Thr Cys Ala Thr Tyr Tyr Lys
20 25 30

Met Asp Gly Ala Ser Asn Val Thr Cys Ile Asn Ser Arg Trp Thr Gly
35 40 45

Arg Pro Thr Cys Arg Asp Thr Ser Cys Val Asn Pro Pro Thr Val Gln
50 55 60

Asn Ala Tyr Ile Val Ser Arg Gln Met Ser Lys Tyr Pro Ser Gly Glu
65 70 75 80

Arg Val Arg Tyr Gln Cys Arg Ser Pro Tyr Glu Met Phe Gly Asp Glu
85 90 95

Glu Val Met Cys Leu Asn Gly Asn Trp Thr Glu Pro Pro Gln Cys Lys
100 105 110

Asp Ser Thr Gly Lys Cys Gly Pro Pro Pro Pro Ile Asp Asn Gly Asp
115 120 125

Ile Thr Ser Phe Pro Leu Ser Val Tyr Ala Pro Ala Ser Ser Val Glu
130 135 140

Tyr Gln Cys Gln Asn Leu Tyr Gln Leu Glu Gly Asn Lys Arg Ile Thr
145 150 155 160

Cys Arg Asn Gly Gln Trp Ser Glu Pro Pro Lys Cys Leu His Pro Cys
165 170 175

Val Ile Ser Arg Glu Ile Met Glu Asn Tyr Asn Ile Ala Leu Arg Trp
180 185 190

Thr Ala Lys Gln
195

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 472 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CACAATATGA CAACCACACT GAATTATCGG GATGGAGAAA AAGTATCTGT TCTTTGCCAA 60

GAAAATTATC TAATTCAGGA AGGAGAAGAA ATTACATGCA AAGATGGAAG ATGGCAGTCA 120

ATACCACTCT GTGTTGAAAA AATTCCATGT TCACAACCAC CTCAGATAGA ACACGGAACC 180

ATTAATTCAT CCAGGTCTTC ACAAGAAAGT TATGCACATG GGACTAAATT GAGTTATACT 240

TGTGAGGGTG GTTTCAGGAT ATCTGAAGAA AATGAAACAA CATGCTACAT GGGAAAATGG 300

AGTTCTCCAC CTCAGTGTGA AGGCCTTCCT TGTAAATCTC CACCTGAGAT TTCTCATGGT 360

GTTGTAGCTC ACATGTCAGA CAGTTATCAG TATGGAGAAG AAGTTACGTA CAAATGTTTT 420

GAAGGTTTTG GAATTGATGG GCCTGCAATT GCAAAATGCT TAGGAGAAAA AT 472

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 385 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GTATCTGTTC TTTGCCAAGA AAATTATCTA ATTCAGGAAG GGGAAGAAAT TACATGCAAA 60

GATGGAAGAT GGCAGTCAAT ACCACTCTGT GTTGAAAAAA TTCCATGTTC ACAACCACCT 120

CAGATAGAAC ACGGAACCAT TAATTCATCC AGGTCTTCAC AAGAAATTTA TGCACATGGG 180

ACTAAATTGA GTTATACTTG TGAGGGTGGT TTCAGGATAT CTGAAGAAAA TGAAACAACA 240

TGCTACATGG GAAAATGGAG TTCTCCACCT CAGTGTGAAG GCCTTCCTTG TAAATCTCCA 300

CCTGAGATTT CTCATGGTGT TGTAGCTCAC ATGTCAGACA GTTATCAGTA TGGAGAAGAA 360

GTTACGTACA AATGTTTTGA AGGTT 385

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 385 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GTATCTGTTC TTTGCCAAGA AAATTATCTA ATTCAGGAAG GAGAAGAAAT TACATGCAAA 60

GATGGAAGAT GGCAGTCAAT ACCACTCTGT GTTGAAAAAA TTCCATGTTC ACAACCACCT 120

CAGATAGAAC ACGGAACCAT TAATTCATCC AGGTCTTCAC AAGAAATTTA TGCACATGGG 180

ACTAAATTGA GTTATACTTG TGAGGGTGGT TTCAGGATAT CTGAAGAAAA TGAAACAACA 240

TGCTACATGG GAAAATGGAG TTCTCCACCT CAGTGTGAAG GCCTTCCTTG TAAATCTCCA 300

CCTGAGATTT CTCATGGTGT TGTAGCTCAC ATGTCAGACA GTTATCAGTA TGGAGAAGAA 360

GTTACGTACA AATGTTTTGA AGGTT 385

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 177 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Asn Cys Ser Met Ala Gln Ile Gln Leu Cys Pro Pro Pro Pro Gln Ile
1 5 10 15

Pro Asn Ser His Asn Met Thr Thr Thr Leu Asn Tyr Arg Asp Gly Glu
20 25 30

Lys Val Ser Val Leu Cys Gln Glu Asn Tyr Leu Ile Gln Glu Gly Glu
35 40 45

Glu Ile Thr Cys Lys Asp Gly Arg Trp Gln Ser Ile Pro Leu Cys Val
50 55 60

Glu Lys Ile Pro Cys Ser Gln Pro Pro Gln Ile Glu His Gly Thr Ile
65 70 75 80

Asn Ser Ser Arg Ser Ser Gln Glu Ser Tyr Ala His Gly Thr Lys Leu
85 90 95

Ser Tyr Thr Cys Glu Gly Gly Phe Arg Ile Ser Glu Glu Asn Glu Thr
100 105 110

Thr Cys Tyr Met Gly Lys Trp Ser Ser Pro Pro Gln Cys Glu Gly Leu
115 120 125

Pro Cys Lys Ser Pro Pro Glu Ile Ser His Gly Val Val Ala His Met
130 135 140

Ser Asp Ser Tyr Gln Tyr Gly Glu Glu Val Thr Tyr Lys Cys Phe Glu
145 150 155 160

Gly Phe Gly Ile Asp Gly Pro Ala Ile Ala Lys Cys Leu Gly Glu Lys
165 170 175

Trp

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 123 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Val Ser Val Leu Cys Gln Glu Asn Tyr Leu Ile Gln Glu Gly Glu Glu
1 5 10 15

Ile Thr Cys Lys Asp Gly Arg Trp Gln Ser Ile Pro Leu Cys Val Glu
20 25 30

Lys Ile Pro Cys Ser Gln Pro Pro Gln Ile Glu His Gly Thr Ile Asn
35 40 45

Ser Ser Arg Ser Ser Gln Glu Ile Tyr Ala His Gly Thr Lys Leu Ser
50 55 60

Tyr Thr Cys Glu Gly Gly Phe Arg Ile Ser Glu Glu Asn Glu Thr Thr
65 70 75 80

Cys Tyr Met Gly Lys Trp Ser Ser Pro Pro Gln Cys Glu Gly Leu Pro
85 90 95

Cys Lys Ser Pro Pro Glu Ile Ser His Gly Val Val Ala His Met Ser
100 105 110

Asp Ser Tyr Gln Tyr Gly Glu Glu Val Thr Tyr
115 120

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 123 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Val Ser Val Leu Cys Gln Glu Asn Tyr Leu Ile Gln Glu Gly Glu Glu
1 5 10 15

Ile Thr Cys Lys Asp Gly Arg Trp Gln Ser Ile Pro Leu Cys Val Glu
20 25 30

Lys Ile Pro Cys Ser Gln Pro Pro Gln Ile Glu His Gly Thr Ile Asn
35 40 45

Ser Ser Arg Ser Ser Gln Glu Ile Tyr Ala His Gly Thr Lys Leu Ser

50 55 60

Tyr Thr Cys Glu Gly Gly Phe Arg Ile Ser Glu Glu Asn Glu Thr Thr
65 70 75 80

Cys Tyr Met Gly Lys Trp Ser Ser Pro Pro Gln Cys Glu Gly Leu Pro
85 90 95

Cys Lys Ser Pro Pro Glu Ile Ser His Gly Val Val Ala His Met Ser
100 105 110

Asp Ser Tyr Gln Tyr Gly Glu Glu Val Thr Tyr
115 120

CLAIMS

1. A method of screening for a cancer comprising the step of detecting the presence of a tumor-associated human complement Factor H-related antigen or a nucleic acid molecule encoding said antigen, said nucleic acid molecule characterized by the ability of said nucleic acid molecule to hybridize under moderate stringency with the primer pair 42M/1040RT (SEQ ID NO:10 and SEQ ID NO:17, respectively) or the primer pair 2910M/3610RT (SEQ ID NO:18 and SEQ ID NO:19, respectively).

2. A method according to claim 1 wherein the method comprises the step of detecting the presence of the antigen.

3. A method according to claim 1 wherein the method comprises the step of detecting the presence of a nucleic acid molecule encoding the antigen.

4. A method according to claim 1, 2 or 3 wherein the cancer is urogenital or renal cancer.

5. A method according to claim 4 wherein the urogenital cancer is bladder, cervical or prostate cancer.

6. A method according to claim 1, 2 or 3 wherein the antigen is further characterized by the ability to bind complement fragment C3b.

7. A method according to claim 1, 2 or 3 wherein the antigen is further characterized by the ability to bind to heparin agarose.

8. A method according to claim 1, 2 or 3 wherein the antigen is further characterized by the presence of a polypeptide with a molecular weight of 138,000 which shifts to a molecular weight of 151,000 in the presence of a disulfide reducing agent, as estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

9. A method according to claim 1, 2 or 3 wherein the antigen is further characterized by at least two of: (1) the ability to bind complement fragment C3b, (2) the ability to bind to heparin agarose, and (3) the presence of a polypeptide with a molecular weight of 138,000 which shifts to a molecular weight of 151,000 in the presence of a disulfide reducing agent, as estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

10. A method of treating a tumor cell comprising the step of modulating a tumor-associated human complement Factor H-related antigen or a nucleic acid molecule encoding said antigen, said nucleic acid molecule characterized by the ability of said nucleic acid molecule to hybridize under moderate stringency with the primer pair 42M/1040RT (SEQ ID NO:10 and SEQ ID NO:17, respectively) or the primer pair 2910M/3610RT (SEQ ID NO:18 and SEQ ID NO:19, respectively).

11. A method according to claim 10 wherein the method comprises the step of modulating the antigen.

12. A method according to claim 10 or 11 wherein the tumor cell is a urogenital or renal tumor cell.

13. A method according to claim 12 wherein the urogenital tumor cell is a bladder, cervical or prostate tumor cell.

14. A method according to claim 10 or 11 wherein the antigen is further characterized by the ability to bind complement fragment C3b.

15. A method according to claim 10 or 11 wherein the antigen is further characterized by the ability to bind to heparin agarose.

16. A method according to claim 10 or 11 wherein the antigen is further characterized by the presence of a polypeptide with a molecular weight of 138,000 which

shifts to a molecular weight of 151,000 in the presence of a disulfide reducing agent, as estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

17. A method according to claim 10 or 11 wherein the antigen is further characterized by at least two of: (1) the ability to bind complement fragment C3b, (2) the ability to bind to heparin agarose, and (3) the presence of a polypeptide with a molecular weight of 138,000 which shifts to a molecular weight of 151,000 in the presence of a disulfide reducing agent, as estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

18. An agent that modulates a tumor-associated human complement Factor H-related antigen or a nucleic acid molecule encoding said antigen, said nucleic acid molecule characterized by the ability of said nucleic acid molecule to hybridize under moderate stringency with the primer pair 42M/1040RT (SEQ ID NO:10 and SEQ ID NO:17, respectively) or the primer pair 2910M/3610RT (SEQ ID NO:18 and SEQ ID NO:19, respectively), for use as a medicament to treat a tumor cell.

19. A composition comprising an agent that modulates a tumor-associated human complement Factor H-related antigen or a nucleic acid molecule encoding said antigen, said nucleic acid molecule characterized by the ability of said nucleic acid molecule to hybridize under moderate stringency with the primer pair 42M/1040RT (SEQ ID NO:10 and SEQ ID NO:17, respectively) or the primer pair 2910M/3610RT (SEQ ID NO:18 and SEQ ID NO:19, respectively), in combination with a pharmaceutically acceptable carrier or diluent.

20. Use of an agent that modulates a tumor-associated human complement Factor H-related antigen or a nucleic acid molecule encoding said antigen, said nucleic acid molecule characterized by the ability of said nucleic acid molecule to hybridize under moderate stringency with the primer pair 42M/1040RT (SEQ ID NO:10 and SEQ ID NO:17, respectively) or the primer pair 2910M/3610RT (SEQ ID NO:18 and SEQ ID NO:19, respectively), for the manufacture of a medicament for the treatment of a tumor cell.

FIG. 1

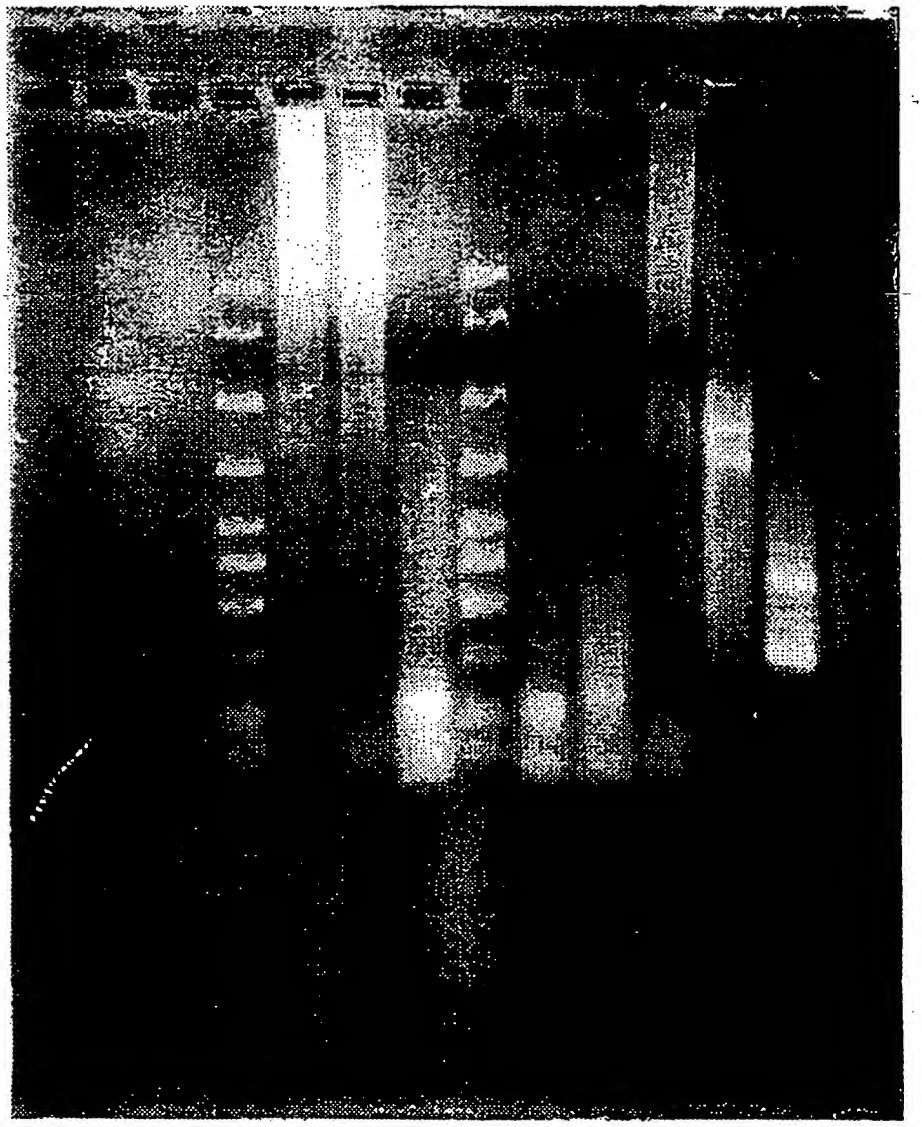

FIG. 2

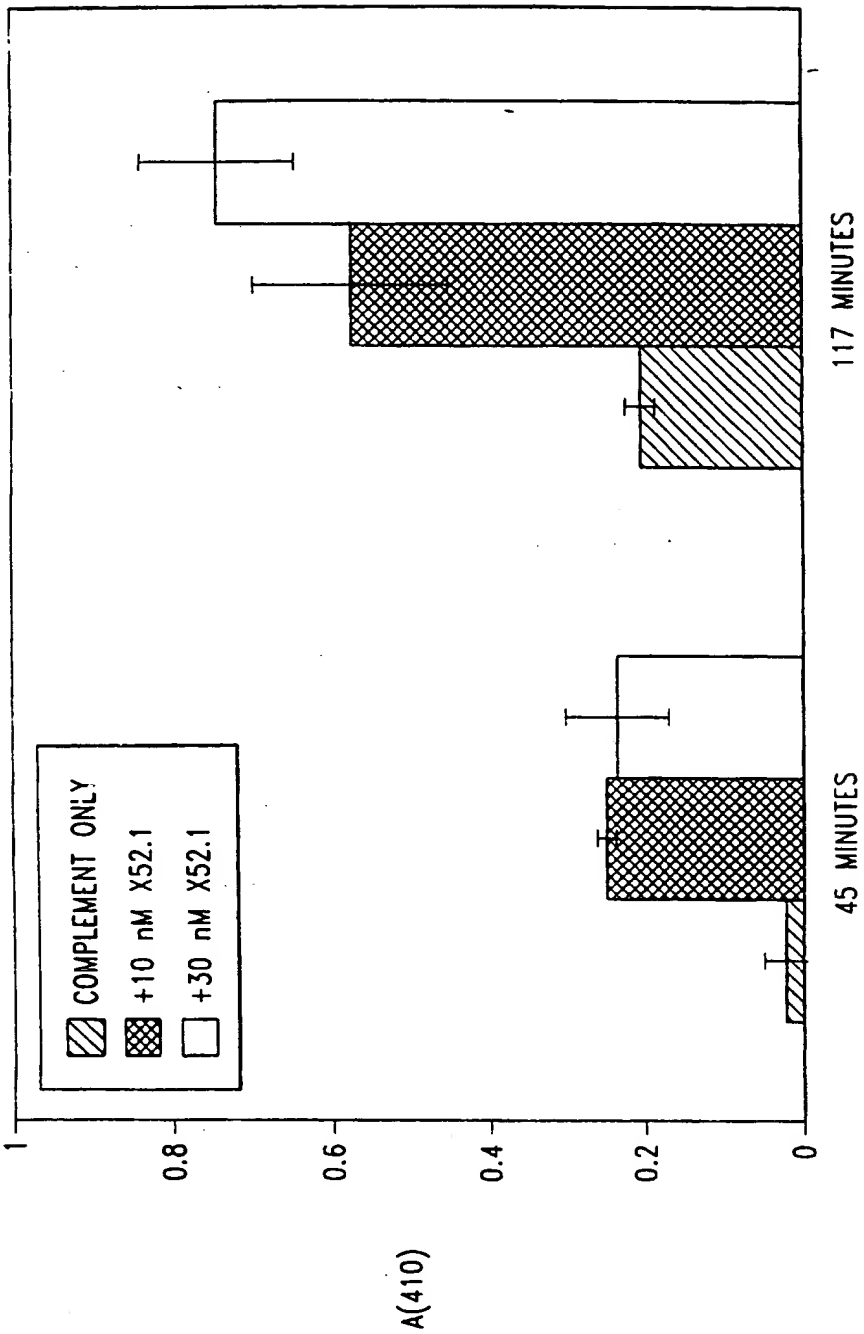

Fig. 3

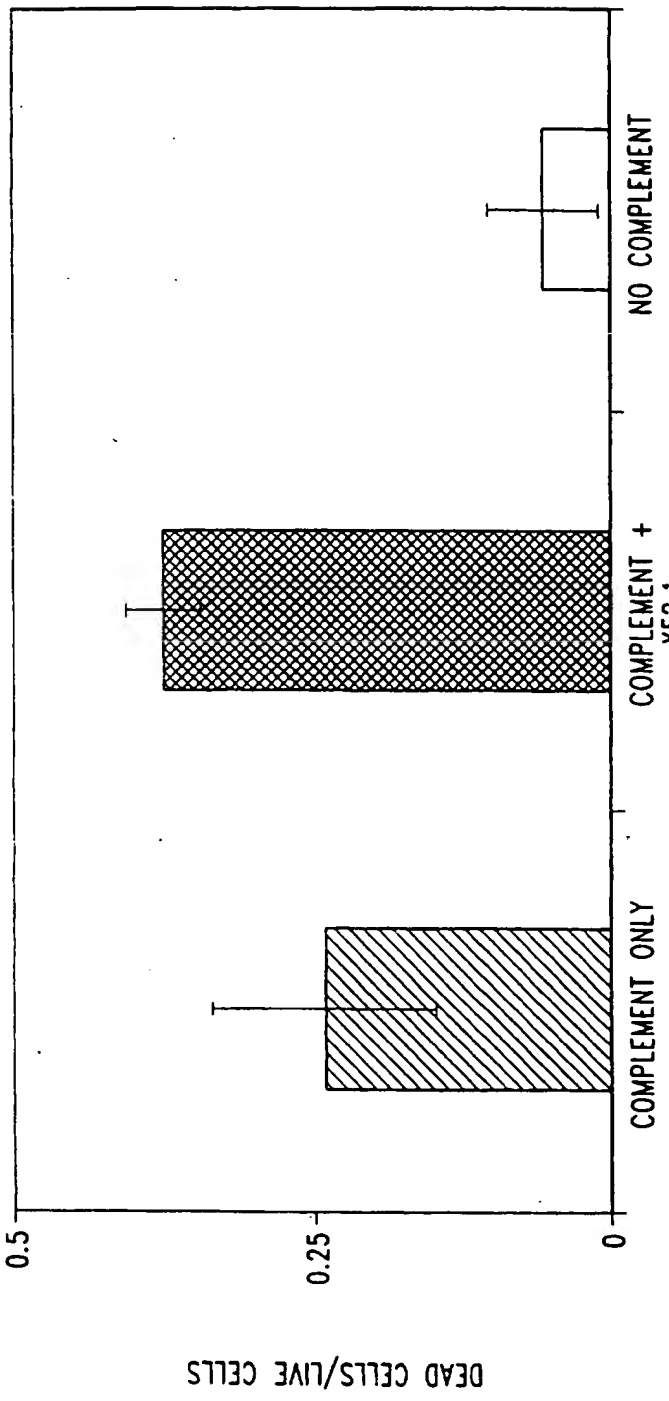

Fig. 4

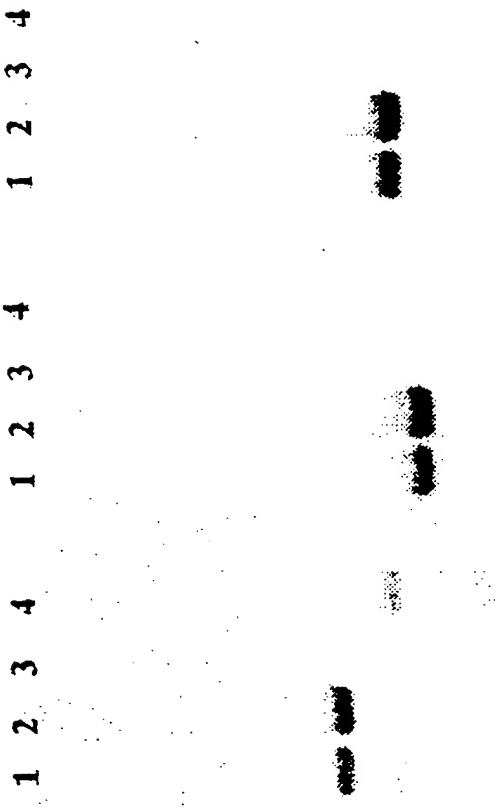

FIG. 5

```
                          420        430        440        450        460        470
                           |          |          |          |          |          |
huCFH 4.4kb cDNAseq       ACATGTAATGAGGGGTATCAATTGCTAGGTGAGATTAATTACCGTGAATGTGACACAGA
pRBB9FH410#2.1            ACATGTAATGAGGGGTATCAATTGCTAGGTGAGATTAATTACCGTGAATGTGACACAGA

                          480        490        500        510        520        530
                           |          |          |          |          |          |
huCFH 4.4kb cDNAseq       TGGATGGACCAATGATATTCCTATATGTGAAGTTGTGAAGTGTTTACCAGTGACAGCAC
pRBB9FH410#2.1            TGGATGGACCAATGATATTCCTATATGTGAAGTTGTGAAGTGTTTACCAGTGACAGCAC

                          540        550        560        570        580        590
                           |          |          |          |          |          |
huCFH 4.4kb cDNAseq       CAGAGAATGGAAAAATTGTCAGTAGTGCAATGGAACCAGATCGGGAATACCATTTTGGA
pRBB9FH410#2.1            CAGAGAATGGAAAAATTGTCAGTAGTGCAATGGAACCAGATCGGGAATACCATTTTGGA

                          600        610        620        630        640
                           |          |          |          |          |
huCFH 4.4kb cDNAseq       CAAGCAGTACGGTTTGTATGTAACTCAGGCTACAAGATTGAAGGAGATGAAGAAATGCA
pRBB9FH410#2.1            CAAGCAGTACGGTTTGTATGTAACTCAGGCTACAAGATTGAAGGAGATGAAGAAATGCA

                     650        660        670        680        690        700
                      |          |          |          |          |          |
huCFH 4.4kb cDNAseq  TTGTTCAGACGATGGTTTTTGGAGTAAAGAGAAACCAAAGTGTGTGGAAATTTCATGCA
pRBB9FH410#2.1       TTGTTCAGACGATGGTTTTTGGGGTAAAGAGAAACCAAAGTGTGTGGAAATTTCATGCA

                     710        720        730        740        750        760
                      |          |          |          |          |          |
huCFH 4.4kb cDNAseq  AATCCCCAGATGTTATAAATGGATCTCCTATATCTCAGAAGATTATTTATAAGGAGAAT
pRBB9FH410#2.1       AATCCCCAGATGTTATAAATGGATCTCCTATATCTCAGAAGATTATTTATAAGGAGAAT

                     770        780        790        800        810        820
                      |          |          |          |          |          |
huCFH 4.4kb cDNAseq  GAACGATTTCAATATAAATGTAACATGGGTTATGAATACAGTGAAAGAGGAGATGCTGT
pRBB9FH410#2.1       GAACGATTTCAATATAAATGTAACATGGGTTATGAATACAGTGAAAGAGGAGATGCTGT

                     830        840        850        860        870        880
                      |          |          |          |          |          |
huCFH 4.4kb cDNAseq  ATGCACTGAATCTGGATGGCGTCCGTTGCCTTCATGTGAAGAAAAATCATGTGATAATC
pRBB9FH410#2.1       ATGCACTGAATCTGGATGGCGTCCGTTGCCTTCATGTGAAGAAAAATCATGTGATAATC

                     890        900        910        920        930        940
                      |          |          |          |          |          |
huCFH 4.4kb cDNAseq  CTTATATTCCAAATGGTGACTACTCACCTTTAAGGATTAAACACAGAACTGGAGATGAA
pRBB9FH410#2.1       CTTATATTCCAAATGGTGACTACTCACCTTTAAGGATTAAACACAGAACTGGAGATGAA

                     950        960        970        980        990        1000
                      |          |          |          |          |          |
huCFH 4.4kb cDNAseq  ATCACGTACCAGTGTAGAAATGGTTTTTATCCTGCAACCCGGGGAAATACAGCCAAATG
pRBB9FH410#2.1       ATCACGTACCAGTGTAGAAATGGTTTTTATCCTGCAACCCGGGGAAATAC

                     1010       1020       1030       1040       1050       1060
                      |          |          |          |          |          |
huCFH 4.4kb cDNAseq  CACAAGTACTGGCTGGATACCTGCTCCGAGATGTACCTTGAAACCTTGTGATTATCCAG
pRBB9FH410#2.1
```

Fig. 6A

```
                        130       140       150       160       170       180
                         |         |         |         |         |         |
CFH-RF3          FTLTGGNVFEYGVKAVYTCNEGYQLLGEINYRECDTDGWTNDIPICEVVKCLPVTAPENG
pRBB9FH410#2.1-RF1                TCNEGYQLLGEINYRECDTDGWTNDIPICEVVKCLPVTAPENG

                        190       200       210       220       230       240
                         |         |         |         |         |         |
CFH-RF3          KIVSSAMEPDREYHFGQAVRFVCNSGYKIEGDEEMHCSDDGFWSKEKPKCVEISCKSPDV
pRBB9FH410#2.1-RF1 KIVSSAMEPDREYHFGQAVRFVCNSGYKIEGDEEMHCSDDGFWGKEKPKCVEISCKSPDV

                        250       260       270       280       290       300
                         |         |         |         |         |         |
CFH-RF3          INGSPISQKIIYKENERFQYKCNMGYEYSERGDAVCTESGWRPLPSCEEKSCDNPYIPNG
pRBB9FH410#2.1-RF1 INGSPISQKIIYKENERFQYKCNMGYEYSERGDAVCTESGWRPLPSCEEKSCDNPYIPNG

                        310       320       330       340       350       360
                         |         |         |         |         |         |
CFH-RF3          DYSPLRIKHRTGDEITYQCRNGFYPATRGNTAKCTSTGWIPAPRCTLKPCDYPDIKHGGL
pRBB9FH410#2.1-RF1 DYSPLRIKHRTGDEITYQCRNGFYPATRGNTAKCTSTGWIPAPRCTLKPCDYP
```

Fig. 6B

```
                                2900       2910       2920       2930       2940       2950
                                 |          |          |          |          |          |
huCFH 4.4kb cDNAseq  CATGGTGTTGTAGCTCACATGTCAGACAGTTATCAGTATGGAGAAGAAGTTACGTACAA
pRBS3FH2910#2.1                          GTCAGACAGTTATCAGTATGGAGAAGAAGTTACGTACAA
pRBS3FH2910#3.1                          GTCAGACAGTTATCAGTATGGAGAAGAAGTTACGTACAA
pRBS3FH2910#4.1

                                2960       2970       2980       2990       3000
                                 |          |          |          |          |
huCFH 4.4kb cDNAseq  ATGTTTTGAAGGTTTTGGAATTGATGGGCCTGCAATTGCAAAATGCTTAGGAGAAAAAT
pRBS3FH2910#2.1      ATGTTTTGAAGGTTTTGGAATTGATGGGCCTGCAATTGCAAAATGCTTAGGAGAAAAAT
pRBS3FH2910#3.1      ATGTTTTGAAGGTTTTGGAATTGATGGGCCTGCAATTGCAAAATGCTTAGGAGAAAAAT
pRBS3FH2910#4.1

                     3010       3020       3030       3040       3050       3060
                      |          |          |          |          |          |
huCFH 4.4kb cDNAseq  GGTCTCACCCTCCATCATGCATAAAAACAGATTGTCTCAGTTTACCTAGCTTTGAAAAT
pRBS3FH2910#2.1      GGTCTCACCCTCCATCATGCATAAAAACAGATTGTCTCAGTTTACCTAGCTTTGAAAAT
pRBS3FH2910#3.1      GGTCTCACCCTCCATCATGCATAAAAACAGATTGTCCCAGTTTACCTAGCTTTGAAAAT
pRBS3FH2910#4.1                                                GTTTGCCTAGCTTTGAAAAT

                     3070       3080       3090       3100       3110       3120
                      |          |          |          |          |          |
huCFH 4.4kb cDNAseq  GCCATACCCATGGGAGAGAAGAAGGATGTGTATAAGGCGGGGTGAGCAAGTGACTTACAC
pRBS3FH2910#2.1      GCCATACCCATGGGAGAGAAGAAGGATTTGTATAAGGCGGGGTGAGCAAGTGACTTACAC
pRBS3FH2910#3.1      GCCATACCCATGGGAGAGAAGAAGGTTTTGTATAAGGCGGGGTGAGCAAGTGACTTACAC
pRBS3FH2910#4.1      GCCATACCCATGGGAGAGAAGAAGGATTTGTATAAGGCGGGGTGAGCCAGTGACTTACAC

                     3130       3140       3150       3160       3170       3180
                      |          |          |          |          |          |
huCFH 4.4kb cDNAseq  TTGTGCAACATATTACAAAATGGATGGAGCCAGTAATGTAACATGCATTAATAGCAGAT
pRBS3FH2910#2.1      TTGTGCAACATATTACAAAATGGATGGAGCCAGTAATGTAACATGCATTAATAGCAGAT
pRBS3FH2910#3.1      TTGTGCAACATATTGCCAAATGGATGGAGCCAGTAATGTAACATGCATTAATAGCAGAT
pRBS3FH2910#4.1      TTGTGCAACATATTACAAAATGGATGGAGCCAGTAATGTAACATGCATTAATAGCAGAT

                     3190       3200       3210       3220       3230       3240
                      |          |          |          |          |          |
huCFH 4.4kb cDNAseq  GGACAGGAAGGCCAACATGCAGAGACACCTCCTGTGTGAATCCGCCCACAGTACAAAAT
pRBS3FH2910#2.1      GGACAGGAAGGCCAACATGCAGAGACACCTCCTGTGTGAATCCGCCCACAGTACAAAAT
pRBS3FH2910#3.1      GGACAGGAAGGCCAACATGCAGAGACACCTCCTGTGTGAATCCGCCCACAGTACAAAAT
pRBS3FH2910#4.1      GGACAGGAAGGCCAACATGCAGAGACACCTCCTGTGTGAATCCGCCCACAGTACAAAAT

                     3250       3260       3270       3280       3290       3300
                      |          |          |          |          |          |
huCFH 4.4kb cDNAseq  GCTTATATAGTGTCGAGACAGATGAGTAAATATCCATCTGGTGAGAGAGTACGTTATCA
pRBS3FH2910#2.1      GCTTATATAGTGTCGAGACAGATGAGTAAATATCCATCTGGTGAGAGAGTACGTTATCA
pRBS3FH2910#3.1      GCTTATATAGTGTCGAGACAGATGAGTAAATATCCATCTGGTGAGAGAGTACGTTATCA
pRBS3FH2910#4.1      GCTTATATAGTGTCGAGACAGATGAGTAAATATCCATCTGGTGAGAGAGTACGTTATCA

                     3310       3320       3330       3340       3350       3360
                      |          |          |          |          |          |
huCFH 4.4kb cDNAseq  ATGTAGGAGCCCTTATGAAATGTTTGGGGATGAAGAAGTGATGTGTTTAAATGGAAACT
pRBS3FH2910#2.1      ATGTAGGAGCCCTTATGAAATGTTTGGGGATGAAGAAGTGATGTGTTTAAATGGAAACT
pRBS3FH2910#3.1      ATGTAGGAGCCCTTATGAAATGTTTGGGGATGAAGAAGTGATGTGTTTAAATGGAAACT
pRBS3FH2910#4.1      ATGTAGGAGCCCTTATGAAATGTTTGGGGATGAAGAAGTGATGTGTTTAAATGGAAACT
```

Fig. 7A-1

```
                          3370       3380       3390       3400       3410       3420
                           |          |          |          |          |          |
huCFH 4.4kb cDNAseq       GGACGGAACCACCTCAATGCAAAGATTCTACAGGAAAATGTGGGCCCCCTCCACCTATT
pRBS3FH2910#2.1           GGACGGAACCACCTCAATGCAAAGATTCTACAGGAAAATGTGGGCCCCCTCCACCTATT
pRBS3FH2910#3.1           GGACGGAACCACCTCAATGCAAAGATTCTACAGGAAAATGTGGGCCCCCTCCACCTATT
pRBS3FH2910#4.1           GGACGGAACCACCTCAATGCAAAGATTCTACAGGAAAATGTGGGCCCCCTCCACCTATT

                          3430       3440       3450       3460       3470       3480
                           |          |          |          |          |          |
huCFH 4.4kb cDNAseq       GACAATGGGGACATTACTTCATTCCCGTTGTCAGTATATGCTCCAGCTTCATCAGTTGA
pRBS3FH2910#2.1           GACAATGGGGACATTACTTCA
pRBS3FH2910#3.1           GACAATGGGGACATTACTTCATTCCCGTTGTCAGTATATGCTCCAGCTTCATCAGTTGA
pRBS3FH2910#4.1           GACAATGGGGACATTACTTCATTCCCGTTGTCAGTATATGCTCCAGCTTCATCAGTTGA

                          3490       3500       3510       3520       3530       3540
                           |          |          |          |          |          |
huCFH 4.4kb cDNAseq       GTACCAATGCCAGAACTTGTATCAACTTGAGGGTAACAAGCGAATAACATGTAGAAATG
pRBS3FH2910#2.1
pRBS3FH2910#3.1           GTACCAATGCCAGAACTTGTATCAACTTGAGGGTAACAAGCGAATAACATGTAGAAATG
pRBS3FH2910#4.1           GTACCAATGCCAGAACTTGTATCAACTTGAGGGTAACAAGCGAATAACATGTAGAAATG

                          3550       3560       3570       3580       3590
                           |          |          |          |          |
huCFH 4.4kb cDNAseq       GACAATGGTCAGAACCACCAAAATGCTTACATCCGTGTGTAATATCCCGAGAAATTATG
pRBS3FH2910#2.1
pRBS3FH2910#3.1           GACAATGGTCAGAACCACCAAAATGCTTACATCCGTGTGTAATATCCCGAGAAATTATG
pRBS3FH2910#4.1           GACAATGGTCAGAACCACCAAAATGCTTACATCCGTGTGTAATATCCCGAGAAATTATG

                     3600       3610       3620       3630       3640       3650
                      |          |          |          |          |          |
Contig# 1                 GAAAATTATAACATAGCATTAAGGTGGACAGCCAAACAGAAGCTTTATTCGAGAACAGG
huCFH 4.4kb cDNAseq       GAAAATTATAACATAGCATTAAGGTGGACAGCCAAACAGAAGCTTTATTCGAGAACAGG
pRBS3FH2910#2.1
pRBS3FH2910#3.1
pRBS3FH2910#4.1           GAAAATTATAACATAGCATTAAGGTGGACAGCCAAACAG
```

Fig. 7A-2

```
                          930       940       950       960       970       980
                           |         |         |         |         |         |
Contig# 1
CFH RF3                   GFRISEENETTCYMGKWSSPPQCEGLPCKSPPEISHGVVAHMSDSYQYGEEVTYKCFE
pRBS3FH2910#2.1-RF2                                                SDSYQYGEEVTYKCFE
pRBS3FH2910#3.1 RF2                                                SDSYQYGEEVTYKCFE
pRBS3FH2910#4.-RF3

                          990      1000      1010      1020      1030      1040
                           |         |         |         |         |         |
CFH RF3                   GFGIDGPAIAKCLGEKWSHPPSCIKTDCLSLPSFENAIPMGEKKDVYKAGEQVTYTCA
pRBS3FH2910#2.1-RF2       GFGIDGPAIAKCLGEKWSHPPSCIKTDCLSLPSFENAIPMGEKKDLYKAGEQVTYTCA
pRBS3FH2910#3.1-RF2       GFGIDGPAIAKCLGEKWSHPPSCIKTDCPSLPSFENAIPMGEKKVLYKAGEQVTYTCA
pRBS3FH2910#4.1-RF3                                LPSFENAIPMGEKKDLYKAGEPVTYTCA

                          1050      1060      1070      1080      1090      1100
                           |         |         |         |         |         |
CFH RF3                   TYYKMDGASNVTCINSRWTGRPTCRDTSCVNPPTVQNAYIVSRQMSKYPSGERVRYQC
pRBS3FH2910#2.1-RF2       TYYKMDGASNVTCINSRWTGRPTCRDTSCVNPPTVQNAYIVSRQMSKYPSGERVRYQC
pRBS3FH2910#3.1 RF2       TYCQMDGASNVTCINSRWTGRPTCRDTSCVNPPTVQNAYIVSRQMSKYPSGERVRYQC
pRBS3FH2910#4.1-RF3       TYYKMDGASNVTCINSRWTGRPTCRDTSCVNPPTVQNAYIVSRQMSKYPSGERVRYQC

                          1110      1120      1130      1140      1150      1160
                           |         |         |         |         |         |
CFH RF3                   RSPYEMFGDEEVMCLNGNWTEPPQCKDSTGKCGPPPPIDNGDITSFPLSVYAPASSVE
pRBS3FH2910#2.1-RF2       RSPYEMFGDEEVMCLNGNWTEPPQCKDSTGKCGPPPPIDNGDITS
pRBS3FH2910#3.1 RF2       RSPYEMFGDEEVMCLNGNWTEPPQCKDSTGKCGPPPPIDNGDITSFPLSVYAPASSVE
pRBS3FH2910#4.1-RF3       RSPYEMFGDEEVMCLNGNWTEPPQCKDSTGKCGPPPPIDNGDITSFPLSVYAPASSVE

                          1170      1180      1190      1200      1210
                           |         |         |         |         |
CFH RF3                   YQCQNLYQLEGNKRITCRNGQWSEPPKCLHPCVISREIMENYNIALRWTAKQKLYSRT
pRBS3FH2910#2.1-RF2
pRBS3FH2910#3.1 RF2       YQCQNLYQLEGNKRITCRNGQWSEPPKCLHPCVISREIM
pRBS3FH2910#4.1-RF3       YQCQNLYQLEGNKRITCRNGQWSEPPKCLHPCVISREIMENYNIALRWTAKQ
```

Fig. 7B

```
                          2540       2550       2560       2570       2580       2590
                           |          |          |          |          |          |
huCFH 4.4kb cDNAseq       CACAATATGACAACCACACTGAATTATCGGGATGGAGAAAAAGTATCTGTTCTTTGCCA
pZS3FH2576#3                                                          GTATCTGTTCTTTGCCA
pZS3FH2576#1/11                                                       GTATCTGTTCTTTGCCA

                          2600       2610       2620       2630       2640       2650
                           |          |          |          |          |          |
huCFH 4.4kb cDNAseq       AGAAAATTATCTAATTCAGGAAGGAGAAGAAATTACATGCAAAGATGGAAGATGGCAGT
pZS3FH2576#3              AGAAAATTATCTAATTCAGGAAGGGGAAGAAATTACATGCAAAGATGGAAGATGGCAGT
pZS3FH2576#1/11           AGAAAATTATCTAATTCAGGAAGGAGAAGAAATTACATGCAAAGATGGAAGATGGCAGT

                          2660       2670       2680       2690       2700       2710
                           |          |          |          |          |          |
huCFH 4.4kb cDNAseq       CAATACCACTCTGTGTTGAAAAAATTCCATGTTCACAACCACCTCAGATAGAACACGGA
pZS3FH2576#3              CAATACCACTCTGTGTTGAAAAAATTCCATGTTCACAACCACCTCAGATAGAACACGGA
pZS3FH2576#1/11           CAATACCACTCTGTGTTGAAAAAATTCCATGTTCACAACCACCTCAGATAGAACACGGA

                          2720       2730       2740       2750       2760       2770
                           |          |          |          |          |          |
huCFH 4.4kb cDNAseq       ACCATTAATTCATCCAGGTCTTCACAAGAAAGTTATGCACATGGGACTAAATTGAGTTA
pZS3FH2576#3              ACCATTAATTCATCCAGGTCTTCACAAGAAATTTATGCACATGGGACTAAATTGAGTTA
pZS3FH2576#1/11           ACCATTAATTCATCCAGGTCTTCACAAGAAATTTATGCACATGGGACTAAATTGAGTTA

                          2780       2790       2800       2810       2820       2830
                           |          |          |          |          |          |
huCFH 4.4kb cDNAseq       TACTTGTGAGGGTGGTTTCAGGATATCTGAAGAAAATGAAACAACATGCTACATGGGAA
pZS3FH2576#3              TACTTGTGAGGGTGGTTTCAGGATATCTGAAGAAAATGAAACAACATGCTACATGGGAA
pZS3FH2576#1/11           TACTTGTGAGGGTGGTTTCAGGATATCTGAAGAAAATGAAACAACATGCTACATGGGAA

                          2840       2850       2860       2870       2880       2890
                           |          |          |          |          |          |
huCFH 4.4kb cDNAseq       AATGGAGTTCTCCACCTCAGTGTGAAGGCCTTCCTTGTAAATCTCCACCTGAGATTTCT
pZS3FH2576#3              AATGGAGTTCTCCACCTCAGTGTGAAGGCCTTCCTTGTAAATCTCCACCTGAGATTTCT
pZS3FH2576#1/11           AATGGAGTTCTCCACCTCAGTGTGAAGGCCTTCCTTGTAAATCTCCACCTGAGATTTCT

                          2900       2910       2920       2930       2940       2950
                           |          |          |          |          |          |
huCFH 4.4kb cDNAseq       CATGGTGTTGTAGCTCACATGTCAGACAGTTATCAGTATGGAGAAGAAGTTACGTACAA
pZS3FH2576#3              CATGGTGTTGTAGCTCACATGTCAGACAGTTATCAGTATGGAGAAGAAGTTACGTACAA
pZS3FH2576#1/11           CATGGTGTTGTAGCTCACATGTCAGACAGTTATCAGTATGGAGAAGAAGTTACGTACAA

                          2960       2970       2980       2990       3000
                           |          |          |          |          |
huCFH 4.4kb cDNAseq       ATGTTTTGAAGGTTTTGGAATTGATGGGCCTGCAATTGCAAAATGCTTAGGAGAAAAAT
pZS3FH2576#3              ATGTTTTGAAGGTT
pZS3FH2576#1/11           ATGTTTTGAAGGTT
```

Fig. 8A

```
                          830        840        850        860        870        880
                           |          |          |          |          |          |
CFH RF3                   NCSMAQIQLCPPPPQIPNSHNMTTTLNYRDGEKVSVLCQENYLIQEGEEITCKDGRWQS
pZS3FH2576#1/11 RF1                                        VSVLCQENYLIQEGEEITCKDGRWQS
pZS3FH2576#3 RF1                                           VSVLCQENYLIQEGEEITCKDGRWQS

                          890        900        910        920        930        940
                           |          |          |          |          |          |
CFH RF3                   IPLCVEKIPCSQPPQIEHGTINSSRSSQESYAHGTKLSYTCEGGFRISEENETTCYMGK
pZS3FH2576#1/11 RF1       IPLCVEKIPCSQPPQIEHGTINSSRSSQEIYAHGTKLSYTCEGGFRISEENETTCYMGK
pZS3FH2576#3 RF1          IPLCVEKIPCSQPPQIEHGTINSSRSSQEIYAHGTKLSYTCEGGFRISEENETTCYMGK

                          950        960        970        980        990       1000
                           |          |          |          |          |          |
CFH RF3                   WSSPPQCEGLPCKSPPEISHGVVAHMSDSYQYGEEVTYKCFEGFGIDGPAIAKCLGEKW
pZS3FH2576#1/11 RF1       WSSPPQCEGLPCKSPPEISHGVVAHMSDSYQYGEEVTY
pZS3FH2576#3 RF1          WSSPPQCEGLPCKSPPEISHGVVAHMSDSYQYGEEVTY
```

Fig. 8B

INTERNATIONAL SEARCH REPORT

Internatic Application No
PCT/US 97/05710

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68 A61K48/00 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. IMMUNOLOGY, pages 4955-4962, XP002038669 OLLERT M. W. ET AL.,: "Classical complement pathway activation on nucleated cells" see the whole document ---	1,2,6, 10-14, 18-20
A	CLIN. EXP. IMMUNOL., vol. 95, - 1994 pages 173-180, XP002038670 DOBBELSTEEN VAN DEN M. ET AL.,: "Regulation of C3 and factor H synthesis of human glomerular mesangial cells by IL-1 and interferon-gamma" see the whole document --- -/--	1-3,6

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
25 August 1997	04. 09. 97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer MUELLER, F

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INTERNATIONAL SEARCH REPORT

Internatic Application No
PCT/US 97/05710

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BIOCHEM. J., vol. 249, - 1988 pages 593-602, XP002038671 RIPOCHE ET AL.,: "The complete amino acid sequence of hu,an complement factor H" see the whole document -----	1-20

2

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int. ational application No.
PCT/US 97/05710

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 10-17
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 10 to 17 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.